

99/115

**USDA LICENSING OF A GENETICALLY ALTERED
VETERINARY VACCINE**

JOINT HEARING

BEFORE THE

NEWARK PUBLIC LIBRARY

SUBCOMMITTEE ON
INVESTIGATIONS AND OVERSIGHT

SEP 25 1986

OF THE

U. S. GOVT. PUBS.
DEPOSITORY 376

**COMMITTEE ON
SCIENCE AND TECHNOLOGY**

AND THE

SUBCOMMITTEE ON DEPARTMENT OPERATIONS,
RESEARCH, AND FOREIGN AGRICULTURE

OF THE

**COMMITTEE ON AGRICULTURE
HOUSE OF REPRESENTATIVES**

NINETY-NINTH CONGRESS

SECOND SESSION

APRIL 29, 1986

[No. 115]

(Committee on Science and Technology)

Serial No. 99-33

(Committee on Agriculture)

Printed for the use of the Committee on Science and Technology
and the Committee on Agriculture



U.S. GOVERNMENT PRINTING OFFICE

WASHINGTON : 1986

61-572 O

For sale by the Superintendent of Documents, Congressional Sales Office
U.S. Government Printing Office, Washington, DC 20402

Y4.Sci 2:

COMMITTEE ON SCIENCE AND TECHNOLOGY

DON FUQUA, Florida, *Chairman*

ROBERT A. ROE, New Jersey
 GEORGE E. BROWN, Jr., California
 JAMES H. SCHEUER, New York
 MARILYN LLOYD, Tennessee
 TIMOTHY E. WIRTH, Colorado
 DOUG WALGREN, Pennsylvania
 DAN GLICKMAN, Kansas
 ROBERT A. YOUNG, Missouri
 HAROLD L. VOLKMER, Missouri
 BILL NELSON, Florida
 STAN LUNDINE, New York
 RALPH M. HALL, Texas
 DAVE McCURDY, Oklahoma
 NORMAN Y. MINETA, California
 MICHAEL A. ANDREWS, Texas
 BUDDY MacKAY, Florida**
 TIM VALENTINE, North Carolina
 HARRY M. REID, Nevada
 ROBERT G. TORRICELLI, New Jersey
 RICK BOUCHER, Virginia
 TERRY BRUCE, Illinois
 RICHARD H. STALLINGS, Idaho
 BART GORDON, Tennessee
 JAMES A. TRAFICANT, Jr., Ohio

MANUEL LUJAN, Jr., New Mexico*
 ROBERT S. WALKER, Pennsylvania
 F. JAMES SENSENBRENNER, Jr.,
 Wisconsin
 CLAUDINE SCHNEIDER, Rhode Island
 SHERWOOD L. BOEHLERT, New York
 TOM LEWIS, Florida
 DON RITTER, Pennsylvania
 SID W. MORRISON, Washington
 RON PACKARD, California
 JAN MEYERS, Kansas
 ROBERT C. SMITH, New Hampshire
 PAUL B. HENRY, Michigan
 HARRIS W. FAWELL, Illinois
 WILLIAM W. COBEY, Jr., North Carolina
 JOE BARTON, Texas
 D. FRENCH SLAUGHTER, Jr., Virginia
 DAVID S. MONSON, Utah

HAROLD P. HANSON, *Executive Director*
 ROBERT C. KETCHAM, *General Counsel*
 REGINA A. DAVIS, *Chief Clerk*
 JOYCE GROSS FREIWALD, *Republican Staff Director*

SUBCOMMITTEE ON INVESTIGATIONS AND OVERSIGHT

HAROLD L. VOLKMER, Missouri, *Chairman*
 MICHAEL A. ANDREWS, Texas
 JAMES A. TRAFICANT, Jr., Ohio
 HARRY M. REID, Nevada
 GEORGE E. BROWN, Jr., California
 RON PACKARD, California
 F. JAMES SENSENBRENNER, Jr.,
 Wisconsin
 SID W. MORRISON, Washington
 GREG SIMON, *Acting Staff Director*
 IRENE GLOWINSKI, *Congressional Science Fellow*
 EILEEN LEE, *Congressional Science Fellow*
 CAROLYN RADABAUGH, *Republican Technical Consultant*

*Ranking Republican Member.

**Serving on Committee on the Budget for 99th Congress.

COMMITTEE ON AGRICULTURE

E (KIKI) DE LA GARZA, Texas, *Chairman*

THOMAS S. FOLEY, Washington,
Vice Chairman

WALTER B. JONES, North Carolina

ED JONES, Tennessee

GEORGE E. BROWN, Jr., California

CHARLES ROSE, North Carolina

JIM WEAVER, Oregon

BERKLEY BEDELL, Iowa

GLENN ENGLISH, Oklahoma

LEON E. PANETTA, California

JERRY HUCKABY, Louisiana

DAN GLICKMAN, Kansas

CHARLES WHITLEY, North Carolina

TONY COELHO, California

TOM DASCHLE, South Dakota

CHARLES W. STENHOLM, Texas

HAROLD L. VOLKMER, Missouri

CHARLES HATCHER, Georgia

ROBIN TALLON, South Carolina

HARLEY O. STAGGERS, Jr., West Virginia

LANE EVANS, Illinois

ROBERT LINDSAY THOMAS, Georgia

JAMES R. OLIN, Virginia

TIMOTHY J. PENNY, Minnesota

RICHARD H. STALLINGS, Idaho

TERRY L. BRUCE, Illinois

EDWARD R. MADIGAN, Illinois,

Ranking Minority Member

JAMES M. JEFFORDS, Vermont

E. THOMAS COLEMAN, Missouri

RON MARLENEE, Montana

LARRY J. HOPKINS, Kentucky

ARLAN STANGELAND, Minnesota

PAT ROBERTS, Kansas

BILL EMERSON, Missouri

SID MORRISON, Washington

STEVE GUNDERSON, Wisconsin

COOPER EVANS, Iowa

GENE CHAPPIE, California

WEBB FRANKLIN, Mississippi

TOM LEWIS, Florida

ROBERT F. (BOB) SMITH, Oregon

LARRY COMBEST, Texas

BILL SCHUETTE, Michigan

PROFESSIONAL STAFF

A. MARIO CASTILLO, *Chief of Staff*

PHILLIP L. FRAAS, *Counsel*

CHARLES HILTY, *Minority Staff Director*

BERNARD BRENNER, *Press Secretary*

SUBCOMMITTEE ON DEPARTMENT OPERATIONS, RESEARCH, AND FOREIGN AGRICULTURE

BERKLEY BEDELL, Iowa, *Chairman*

GEORGE E. BROWN, Jr., California

HARLEY O. STAGGERS, Jr., West Virginia

LEON E. PANETTA, California

CHARLES HATCHER, Georgia

TIMOTHY J. PENNY, Minnesota

THOMAS S. FOLEY, Washington

HAROLD L. VOLKMER, Missouri

PAT ROBERTS, Kansas

SID MORRISON, Washington

STEVE GUNDERSON, Wisconsin

COOPER EVANS, Iowa

LARRY COMBEST, Texas

CONTENTS

WITNESSES

April 29, 1986:

	Page
Dr. Saul Kit, Baylor College of Medicine and Novagene, Inc., accompanied by Gordon Kit, Esq., attorney; and Dr. Michael J. Bartkoski, Jr., vice president for operations, Biologics Corp., Division of TechAmerica Group, Inc., Animal Health Division, accompanied by Edward J. Allera, Esq., counsel, Finley, Kumble, Wagner, Heine, Underberg, Manley & Casey	6
Dr. Orville G. Bentley, Assistant Secretary for Science and Education, accompanied by Alan Tracy, Acting Assistant Secretary, Marketing and Inspection Services, and Thomas M. Walsh, Assistant General Counsel, OGC, USDA	195
Jeremy Riskin, president, Foundation on Economic Trends	279

APPENDIXES

1. Letter to Committee on Science and Technology from Cornell University ..	289
2. Baylor College of Medicine IBC report	290
3. Texas A&M IBC letter to NIH	293
4. Chronology of events concerning the licensure of the pseudorabies vaccine	297
5. USDA followup questions and answers for the record	302
6. TechAmerica followup questions and answers for the record	312
7. Label sketch for experimental vaccine	316
8. Veterinary biologics production and test report for pseudorabies virus vaccine	317
9. USDA/TechAmerica correspondence re: pseudorabies vaccine (in chronological order)	318
10. Baylor College of Medicine (Dr. Saul Kit)/Biologics Corp. (TechAmerica) correspondence re pseudorabies vaccine	328

USDA LICENSING OF A GENETICALLY ALTERED VETERINARY VACCINE

TUESDAY, APRIL 29, 1986

HOUSE OF REPRESENTATIVES, COMMITTEE ON SCIENCE AND
TECHNOLOGY, SUBCOMMITTEE ON INVESTIGATIONS AND
OVERSIGHT, AND COMMITTEE ON AGRICULTURE, SUBCOM-
MITTEE ON DEPARTMENT OPERATIONS, RESEARCH, AND
FOREIGN AGRICULTURE,

Washington, DC.

The subcommittees met, pursuant to call, at 9:40 a.m., in room 2318, Rayburn House Office Building, Hon. Harold L. Volkmer (chairman of the Subcommittee on Investigations and Oversight) presiding.

Present: Representatives Volkmer, Bedell, Penny, Packard, Sensenbrenner, Morrison, and Evans of Iowa.

Also present: Representative Daub.

Mr. VOLKMER. Good morning. The Subcommittee on Investigations and Oversight will come to order. I am pleased today to welcome members of the Agriculture Subcommittee on Department Operations, Research, and Foreign Agriculture, and its chairman, Congressman Berkely Bedell of Iowa.

The interested participation of Mr. Bedell and the DORFA Subcommittee is a natural result of the fact that nearly all of the earliest products of biotechnology are intended to benefit agriculture.

The great potential of biotechnology to enhance our agriculture output is one of the reasons the Investigations and Oversight Subcommittee has held a series of hearings on developments in biotechnology.

An equally important reason for these hearings is a need to insure that Federal regulation of biotechnology is fair, consistent, and designed to insure that biotechnology products are developed and used in a safe manner. Each Federal agency that deals with biotechnology products has developed guidelines to regulate the manner in which genetically engineered products will be reviewed and approved for release. New guidelines are expected in the next few weeks. When these are published, the Investigations and Oversight Subcommittee will hold a hearing to examine the proposed coordinated frame work for Federal review of biotechnology.

Today's hearing focuses on the events surrounding the licensing of a genetically engineered veterinary drug in this case a vaccine called OMNIVAC that prevents pseudorabies, a deadly disease found in swine and cattle.

To briefly review the facts—in April of 1985, the Department of Agriculture allowed field trials of OMNIVAC in animals in several States. USDA did not consider the vaccine to be a recombinant organism at the time of the field test, even though published literature and the company's own application referred to the fact that the vaccine was created through recombinant DNA technologies. In November 1985, after completion of field trials, USDA classified the vaccine as a recombinant organism.

On April 8, 1986, USDA announced that TechAmerica group voluntarily suspended sales of the vaccine pending publication of an environmental assessment as required by the National Environmental Protection Act. The environmental assessment was published on April 22 and sales were resumed. Jeremy Rifkin of the Foundation on Economic Trends, one of our witnesses today, has since filed suit against USDA to take the vaccine off the market.

Last December 1985, before a hearing of this subcommittee, USDA representatives testified that guidelines were in place to govern the review and regulation of proposals to use or release genetically engineered organisms in the environment, including recombinant organisms. These guidelines include review by USDA's Agriculture Recombinant DNA Research Committee, otherwise known as the ARRC. The subcommittee has learned that USDA's ARRC did not formally review the application to license this vaccine.

Our review of these events and the hearing today will focus on two areas. First, did the developers of this vaccine play fair with the USDA?

In March of this year, this subcommittee held a hearing to investigate allegations that a developer of genetically engineered bacteria to prevent frost on strawberry plants had conducted unauthorized experiments and submitted invalid data to the EPA. EPA has since withdrawn the company's experimental use permit.

We will examine whether the inventor of the vaccine, Dr. Saul Kit of Baylor, and the company that licensed the vaccine, TechAmerica Group, Inc., played fair by following all applicable rules governing testing of the vaccine and whether they provided USDA with sufficient information to allow USDA to determine prior to field testing whether the vaccine qualified as a recombinant organism.

The second question we will examine today is: Did USDA play fair with the company and the public?

Federal regulation of biotechnology is necessary to insure that the public and the environment are protected from possible harm. But if USDA did not follow its own guidelines in a timely manner, then it has not played fair to the detriment of the company that developed the vaccine and the farmers who need it.

Now, in our proposed original hearing, we had planned to have Dr. John Jordan, who is chairman of the USDA ARRC Committee; Dr. Shibley, who is Veterinary—former Veterinary Services; and Mr. Bert Hawkins of APHIS, Animal and Plant Health Inspection Service.

However, the committee has now been informed by the General Counsel of USDA that because of the lawsuit that has been filed by Mr. Rifkin that they won't be here to testify today. We will, and we

plan to, pursue the matter in the future, and for the purpose of, as I was saying, determining whether or not USDA plans to follow its own Guidelines in the future on licensing of biotechnology developed products. However, today we will have Dr. Bentley and Mr. Tracy testify from USDA and we will try and pursue that matter through them.

Now, in preparation for these hearings, the subcommittee staff made extensive requests for documents from TechAmerica, who replied fully and in a timely manner, and we wish to thank them for that. I wish to commend TechAmerica for its cooperation; and I will urge others, such as USDA, to follow its example in future hearings.

At this time I would like to recognize the distinguished gentleman from Iowa, Congressman Berkley Bedell, for any opening statement he may wish to make.

Mr. BEDELL. Thank you, Mr. Chairman.

On behalf of the Subcommittee on Department Operations, Research, and Foreign Agriculture, I want to thank you, Mr. Chairman, for asking us to join the Investigations and Oversight Subcommittee in reviewing the apparent weaknesses in the Department of Agriculture's program for regulating biotechnology.

The incident that gave rise to today's hearing is especially disturbing, because it comes after we had received assurances from USDA officials that following a sustained period of public criticism, the Department finally has its biotechnology review structure in order.

The continued fumbling calls into question USDA's basic ability to manage its biotechnology responsibilities without further direction and guidance from the Congress. At stake here is much more than just the jurisdictional turf of agencies within the Department, or USDA's share of the Federal Government's role in regulating biotechnology.

The larger issues are really public confidence in genetic engineering as a promising new technology and the potential that biotechnology holds for improving farm income.

Certainly, incidents such as this one undermine public confidence in the ability of regulatory officials to prevent the release of a potentially harmful genetically engineered product and understandably so. During these early stages of bringing a new technology to the marketplace, we should be particularly careful to assure that proper safeguards are in place and that established procedures are followed. Failure to do so will likely invite public demands that restrictions be put in place that could slow the responsible development of biotechnology.

In agriculture, farmers themselves stand to pay the cost if progress on biotechnology is sidetracked at this critically important point. The live virus vaccine for pseudorabies is a case in point. Pseudorabies continues to inflict multimillion dollar losses on pork producers, including many in my own State of Iowa. This new vaccine is promising because it provides effective control of the disease, and unlike the old vaccine, apparently still allows producers to accurately detect which animals have the disease and which do not.

Today I look forward to hearing not simply what went wrong at USDA, but what concrete measures the Department intends to take to make sure that this mishap is not repeated.

Again, I speak for my entire subcommittee in thanking you for letting us join you in these hearings, Mr. Chairman.

Mr. VOLKMER. I thank the gentleman from Iowa for being here and at this time I will recognize the ranking minority member, the gentleman from California, Congressman Packard.

Mr. PACKARD. Thank you, Mr. Chairman.

The pseudorabies vaccine that we have heard so much about during the past several months appears to be a remarkable achievement. The potential savings of millions of dollars to our farmers is good news and it is just a hint of the benefits biotechnology promises for the future.

The Science and Technology Committee and in particular the Subcommittee on Investigations and Oversight has closely monitored biotechnology research over the years. Now that this research is beginning to show encouraging results, there is a need to insure that Federal guidelines are in place to bring these products safely and efficiently to the marketplace.

I am very worried about the Department of Agriculture's review in handling of the licensing of Omnivac, a genetically altered live vaccine—or live virus vaccine.

Several months ago, this subcommittee heard testimony from the USDA who clearly stated that USDA had prepared to hand—was prepared to handle and review applications for field testing and licensing of genetically engineered organisms.

While the guidelines have been essentially developed and put into place, it appears as though USDA—their right hand doesn't seem to know precisely what their left hand is doing. However, I will give USDA the benefit of any doubt until I hear today's testimony and are able to followup with other aspects of this particular problem.

In addition to my concern about regulatory procedures, USDA and TechAmerica group followed in the licensing of the vaccine, I am equally concerned about USDA's apparent disregard for public concern about health and environmental safety.

The biotechnology industry is in its infant stages and great care must be taken to address realistic fears and uncertainties about this new technology.

Unfortunately, those most hurt by such actions is the biotechnology industry and American competitiveness. Actions or inactions taken now will have lasting effects on the competitiveness and the economic future of the industry.

The Federal regulatory framework for biotechnology can only be effective if both the industry and the Government fully and responsibly cooperate.

I commend you, Mr. Chairman, for calling this hearing and I appreciate the time and cooperation of our witnesses, and look forward to their testimony.

Mr. VOLKMER. I thank the gentleman for his statement.

Our first panel today consists of Dr. Saul Kit and Dr. Michael Bartkoski.

Dr. Kit is the inventor of the pseudorabies vaccine, Omnivac, and is the head of the division of biochemical virology—virology, at the Baylor College School of Medicine.

Dr. Bartkoski is vice president for operations at TechAmerica Group, Inc., a company that has a license to market Omnivac.

At this time, before proceeding with the panel, I would like to recognize the gentleman from Nebraska, Mr.—Congressman Daub.

Mr. DAUB. Mr. Chairman, first let me say that I am very pleased, considering the controversy which is surrounding this particular matter, that you and your committee saw fit to hold another hearing to inquire—and I commend you for the joint nature of the hearings, and I think that it certainly is going to go a long way to resolve some of the questions that some may have. The soundness and safety, with respect to the public health, are critical. You have always discharged your responsibilities in that regard in the highest fashion, and all of your colleagues in the Congress recognize that and appreciate your leadership.

Pseudorabies is a situation, a problem, for my farmers in Nebraska as well. I had the privilege of being involved in a livestock feed manufacturing company for a period of 10 years before coming to the Congress. I am most acquainted with the nature of it and the great cost that it brings to the pork-producing industry.

This particular research and development activity and biogenetic and genetic engineering is a pioneering effort on the part of Dr. Kit and on the part of the company headquartered for this particular manufacturing operation in Omaha, NE—a company known as TechAmerica. It is a company with which I am familiar because their predecessor company is a company for whom I did legal work. I know the players. I rate them to be competent in their staff work and very careful as a team. I am proud of their work and America is proud of their work in this pioneering effort of a great contribution in the offering to American agriculture.

I want to say to you, Mr. Chairman, that I believe that they, too, as you commended them for their cooperation with the committee, were acting in the public interest as is this committee today. And I want to commend particularly Dr. Bartkoski, a witness on your first panel, for being here today and for essentially standing up for the great potential that I know the Science and Technology Committee recognizes can be in the offing for America and in this case particularly for the livestock producers of our country.

I want to thank you very much for this opportunity to introduce one of your first witnesses in this hearing.

Mr. VOLKMER. I thank the gentleman from Nebraska.

At this time, the Chair will ask Dr. Kit to proceed with his testimony. I have reviewed the testimony and the documents that I appreciate you forwarding to the committee. You may either summarize your testimony or review it in full. The document will be made a part of the record, at this point in the record, in full.

Dr. Kit.

STATEMENTS OF DR. SAUL KIT, BAYLOR COLLEGE OF MEDICINE AND NOVAGENE, INC., ACCOMPANIED BY GORDON KIT, ESQ., ATTORNEY; AND DR. MICHAEL J. BARTKOSKI, JR., VICE PRESIDENT FOR OPERATIONS, BIOLOGICS CORP., DIVISION OF TECHAMERICA GROUP, INC., ANIMAL HEALTH DIVISION, ACCOMPANIED BY EDWARD J. ALLERA, ESQ., COUNSEL, FINLEY, KUMBLE, WAGNER, HEINE, UNDERBERG, MANLEY & CASEY

Dr. Kit. Thank you very much, Mr. Chairman.

Mr. Chairman, and members of the committee:

My name is Dr. Saul Kit. And for nearly 25 years I have been a professor and head of the division of biochemical virology at the Baylor College of Medicine in Houston.

However, I am not here as a representative of the Baylor College of Medicine, but rather, as the chairman of the Scientific Advisory Board of NovaGene, Inc., a small, new research and development company in Houston.

I am also here to communicate the perspective of a scientist who has been working in the laboratory for over 35 years.

I want to make five points in considering the licensing of vaccines developed by NovaGene for veterinary use in combating pseudorabies in pigs and herpes virus in cattle.

The first is the role of NovaGene. The research was entirely funded by the Houston-based company, NovaGene, Ltd. The development of the vaccine was carried out in my laboratory at the Baylor College of Medicine. No Federal funds were involved. NovaGene, Inc. was formed by my oldest son, Dr. Malon Kit, a physician-scientist, and Robert A. Axelrad, an attorney in Houston. Malon Kit is president of NovaGene, Inc. and NovaGene, Inc. is the general partner of NovaGene, Ltd.

U.S. Patent No. 4,514,497, issued on April 30, 1985, as a result of our research. This patent covers a novel pseudorabies vaccine and is jointly owned by NovaGene, Ltd., and the Baylor College of Medicine.

TechAmerica Group, Inc. of Elwood, KS is the licensee of NovaGene, Ltd. and the Baylor College of Medicine under U.S. Patent No. 4,514,497. And after extensive testing of the vaccine by the TechAmerica Group under the review of the U.S. Department of Agriculture, the U.S. Department of Agriculture issued a product license to TechAmerica to market the pseudorabies vaccine, and the pseudorabies vaccine, as you know, is marketed under the name of Omnivac-PRV.

Now, a second patent, that is, U.S. Patent 4,569,840, issued on February 11, 1986, as a result of our research. This patent covers a novel bovine herpesvirus vaccine and is jointly owned by NovaGene, Ltd. and the Baylor College of Medicine.

Patents are pending on second-generation vaccines for both pseudorabies virus and bovine herpesvirus.

Now, with regard to the vaccine.

Pseudorabies in swine and bovine herpesvirus in cattle are highly destructive within our Nation's livestock industry, resulting in an estimated \$55 million in annual losses, at least. As much as 14 percent of the 70 million swine in the United States are already infected with pseudorabies, which causes reduced growth, infertile-

ity, abortion and death. And bovine herpesvirus infects about 65 percent of the cattle in the United States.

Our vaccine is the first modified-live virus of its kind. Although all modified live virus vaccines are genetically altered, previous vaccines were developed by a hit or a miss process, much like a blind dentist attempting to do a root canal. If he drills long enough on enough teeth, he may eventually find the right one, but it will be a long and painful process.

On the other hand, our work was like the dentist who knows the right tooth, and the benefit of x rays and good vision. That is, we knew our target, a gene named thymidine kinase, which when inactivated makes the virus safer, while still protective against pseudorabies disease.

Our vaccine is safer than previous vaccines, not only because it contains a deletion in the thymidine kinase gene, but also because it cannot revert to an infectious form.

In contrast, conventional live virus vaccines can revert to an infectious form. And even if our vaccine did revert, it would not—it would revert to a commercially used pseudorabies vaccine since in the development of our vaccine, we employed an existing vaccine as the starting material.

Another advantage of our vaccine is that it is distinguishable for epidemiological purposes, from field strains of pseudorabies and other vaccine strains of pseudorabies.

Now, third, the testing.

The first tests of our vaccine were done in my laboratory at the Baylor College of Medicine with mice and rabbits, under controlled conditions, with the full approval of the Institutional Biosafety Committee.

The second phase of testing was done by Dr. Eugene Pirtle, the senior scientist in pseudorabics at the U.S. Department of Agriculture's National Animal Disease Center in Ames, IA. Dr. Pirtle carried out the first pig safety experiments. In these experiments, he used 100 million infectious units of the vaccine per pig. The vaccine's effectiveness was demonstrated by challenging the treated pigs with more than 500 million infectious units of an illness-causing pseudorabies, yet, none of the pigs developed signs of disease. Normally, 10,000 units of a virulent strain can kill a pig in 3 to 5 days.

Now, subsequently we were asked to use the vaccine to help to stop an outbreak of pseudorabies on a quarantined Texas farm. This proposal was discussed for 3 months with veterinarians at Texas A&M University and with Dr. John Holcombe, the animal health commissioner of the State of Texas. Dr. Holcombe approved the experiment to use the vaccine in order to attempt to prevent the spread of the pseudorabies outbreak. The State of Texas and NovaGene, Limited jointly financed the test. We have followed the herd for over 2 years and there is no sign of pseudorabies disease on this farm.

We also carried out small pilot experiments on lambs and calves. And these experiments demonstrated that the vaccine is safe in lambs and calves and that the vaccine virus is not transmitted from animal to animal. It should be noted that even if the virus had been transmitted, it would not cause illness because the thymi-

dine kinase gene, that is, the pseudorabies gene that facilitates replication and re-infection, has been removed from the virus.

Now, fourthly, the recombinant DNA and the environment. Our work was done in full compliance with the Guidelines of Veterinarian Services No. 800.68* which states, and I quote, "in normal husbandry and laboratory practices, veterinary biological products are not considered to be released into the environment." [The material referred to above follows:]



United States
Department of
Agriculture

Animal and
Plant Health
Inspection Service

Washington, DC
20250

December 4, 1984

VETERINARY SERVICES MEMORANDUM NO. 800.68

Subject: New Biotechnology for Preparation of Animal Biological Products

To : Biologics Licensees and Applicants
National Veterinary Services Laboratories
Regional Directors
Biologics Specialists
Area Veterinarians in Charge

I. PURPOSE

The purpose is to establish policies and procedures for use of new biotechnological procedures for preparation of veterinary biological products subject to licensure under the provisions of Title 9, Code of Federal Regulations (9 CFR), Parts 101 to 117.

II. POLICIES AND PROCEDURES

A. General

Veterinary biological products prepared using new biotechnological procedures such as recombinant DNA, chemical synthesis, or hybridoma technology will be treated as analogous to products prepared by conventional techniques. A separate license will be required for each such product even if the product is identical in molecular or chemical structure to a naturally occurring substance or a conventionally prepared product.

The unlimited number and kinds of products that may result from these new biotechnological procedures make it impossible to define all requirements in specific terms. Each product must be evaluated individually to determine what will be necessary to establish its purity, safety, potency, and efficacy. Special assays, preferably using *in vitro* methods, may be required for potency and stability determinations. Additional tests may be required to assure safety, especially when live microorganisms are present in the biological product.

VS Memorandum No. 800.68

B. Recombinant Products

1. Guidelines

The United States Department of Agriculture (USDA) requires recipients of research grants and manufacturers of veterinary biological products using recombinant DNA technology to follow guidelines provided by the National Institutes of Health (NIH). These guidelines are also appropriate for development and production of animal biological products.

"Guidelines for Research Involving Recombinant DNA Molecules" and "Recombinant DNA Research; Physical Containment Recommendations for Large-Scale Use of Organisms Containing Recombinant DNA Molecules" have been published and are periodically updated in the Federal Register. These two documents, along with "Administrative Practices Supplement to the NIH Guidelines for Research Involving Recombinant DNA Molecules" published by NIH, outline the current policy of NIH concerning recombinant DNA research and the procedures required to contain recombinant microorganisms when used in large-scale production. Copies of these documents may be obtained upon request from the Office of Recombinant DNA Activities, NIH, Room 4A52, Building 31, Bethesda, MD 20205.

2. Recombinant DNA Technology

The technology encompasses the isolation, characterization, and insertion of foreign DNA into vectors for the production of foreign gene products in suitable expression systems.

a. Foreign DNA

The genetic information coding for the product of interest is referred to as foreign DNA. It consists of a naturally occurring or chemically synthesized nucleotide sequence known to code for a functional gene product. The specific cloned nucleotide segment coding for the desired product must be defined in data supporting each license application. These data must also include a description of antigen or DNA source material, the nucleotide sequence, and the restriction endonuclease digestion map.

b. Vectors

A vector is a cloning vehicle which provides a suitable origin of replication necessary for production of foreign DNA. Such replicons include plasmids, bacteriophage, or viruses such as vaccinia, bovine papillomavirus, adenoviruses, or SV40. A restriction endonuclease map of the vector construct describing structural genes, regulatory or promoter regions, insert orientation, and a description of readily detectable phenotypic traits on host cells will be required as supporting data.

c. Expression Systems

Production of functional gene products depends on the efficient expression of cloned DNA-vector complexes in suitable host microorganisms such as Escherichia coli, Bacillus subtilis, or Saccharomyces cerevisiae. Tissue culture cells may also be used as expression systems for replication of vectors. The mechanism of transfer, copy number, and the physical state of the constructed vector inside the host cell, integrated or extrachromosomal, should be described.

3. Chemically Synthesized Antigens

When the product consists of chemically synthesized polypeptides, the appropriate amino acid sequences will mimic the antigenic site or epitope found in the native antigen. Supporting data shall include type, degree, and persistence of the immune response following administration of the synthetic peptide. Procedures used to increase and prolong the antibody response such as coupling to carrier proteins or addition of adjuvants, must also be described.

4. Master Seeds

a. Recombinant derived bacterial or viral seed stocks used to prepare veterinary biological products must meet established procedures used to certify Master Seeds for conventionally prepared biological products (9 CFR 101.7).

b. Tissue culture-propagated cells from vertebrate animals used for vector propagation and antigen production must meet the requirements of 9 CFR 113.51 or 113.52.

c. If the gene product in any preparation has been adequately characterized, use of newly constructed Master Seed vectors may be permitted under abbreviated immunogenicity test procedures, subject to reevaluation for safety.

5. Product and Serial Release

Each Outline of Production shall be prepared in accordance with 9 CFR 114.9. Outlines must include procedures to ensure consistency in production and recovery of specific antigenic material. Recovery procedures must include the removal of excessive antibiotic levels (9 CFR 114.10) and undesirable fermentation byproducts such as bacterial endotoxins.

In addition to the serial release tests for purity, safety, and potency, additional antigenic characterization such as electrophoretic mobility or immunoelectrophoresis may be required to demonstrate consistent gene expression.

VS Memorandum No. 800.68

4

C. Monoclonal Antibody Products

1. The specificity and potency of monoclonal antibody products will be compared with that of similar polyclonal antibody products. The specificity and sensitivity of monoclonal antibody products must be at least equal to that of antibody products of traditional polyclonal nature.

2. Monoclonal antibody products must be derived from Master Cell Stocks which meet the requirements of 9 CFR 113.52. Description of cell cloning procedures and preparation and characterization of cell passages must also be provided.

3. The Outline of Production must provide a description of all processes including scale-up, ascites fluid or cell culture supernate preparation, purification, concentration, and inactivation. Mouse colonies must be screened to demonstrate freedom from adventitious agents, especially those detected by the mouse antibody production (MAP) test. If the MAP test discloses the presence of murine leukemia viruses or other adventitious agents, the product shall not be released unless inactivation procedures approved by Veterinary Services have been conducted.

The immunologic specificity of the monoclonal antibody product must be fully described, and methods used to characterize the product and to evaluate all production serials prior to release must be included in the filed Outline of Production.

III. ENVIRONMENTAL CONSIDERATIONS

Under the present NIH guidelines, the deliberate release of any organism containing recombinant DNA into the environment is subject to review and approval by appropriate Federal agencies. In normal husbandry and laboratory practices, veterinary biological products are not considered to be released into the environment. In the event that a veterinary biological product would be considered to be released into the environment, the issuance of a license or import permit may require an environmental impact statement and interagency approval.

K. R. Hook
K. R. Hook
Acting Deputy Administrator
Veterinary Services

Now, also, in January of 1986, the Environmental Protection Agency submitted a report at the Paris meeting of the Organization of Economic Cooperation and Development. The report deals with the safety considerations for commercial and environmental applications of organisms derived by recombinant DNA technology.

The report states, and I quote: "genetic engineering of organisms has existed long before the ability to modify directly the cell materials. It dates from man's recognition that animals and crop plants could be selected and crossed to produce the desired phenotype" And then there are other paragraphs and the conclusion is: "there is no scientific justification for singling out recombinant DNA work as for a special regulatory regime over and above that which is already established to regulate traditional biotechnology."

Now, lastly, the second generation vaccine, which I reported about 2 or 3 weeks ago here in Washington. Our work on a second-generation virus vaccine was carried out in response to the need to distinguish by a simple blood test, a vaccinated animal from an infected animal. Our second-generation pseudorabies vaccine can safely and simply distinguish between vaccinated pigs and infected pigs.

And my conclusions are: Our hope is to eliminate pseudorabies and bovine herpes from the farms of the United States and other countries through vaccination programs. It will save the livestock industry many millions of dollars, and our Nation and the world cannot have healthy people without a healthy food supply. We hope we can help farmers protect the health of the meat supply through the vaccines we have developed.

Now, we may also contribute something to protecting our wildlife endangered species. We have been asked by the University of Florida at Gainesville to collaborate in tests to save the Florida panther, the State animal. There are fewer than 50 still alive. These panthers feed on wild pigs, 35 percent of which are infected with pseudorabies virus.

We are concerned that pseudorabies virus may be a cause of death of some panthers because pseudorabies virus causes fatal infections in cats. We believe that our vaccine can also be used to help to save the endangered Florida panther.

And most of all, we believe that our research and its applications to vaccine development will provide data applicable to the design of human vaccines, and that this work will contribute to the economic well-being of the agricultural industry and the overall health of the human population.

Thank you very much, sir.

[The prepared statement of Dr. Kit follows:]

Testimony of Saul Kit, Ph.D.
 Before the Subcommittee on Investigations and Oversight
 Committee on Science and Technology
 U.S. House of Representatives
 April 29, 1986

A SUMMARY

Five points are important in considering the licensing of vaccines developed by NovaGene for veterinary use in combating pseudorabies in pigs and herpesvirus in cattle.

1. The Role of NovaGene.

The research was entirely funded by the Houston based company, NovaGene, Ltd. Development of the vaccine was carried out in my laboratory at the Baylor College of Medicine. No federal funds were involved. NovaGene, Inc., was formed by my son, Dr. Malon Kit, M.D. and Robert A. Axelrad, Esq. Dr. Malon Kit is President of NovaGene, Inc. NovaGene, Inc. is the General Partner of NovaGene, Ltd.

U.S. Patent No. 4,514,497 issued on April 30, 1985 as a result of our research. This patent covers a novel pseudorabies vaccine and is jointly owned by NovaGene, Ltd. and Baylor College of Medicine.

TechAmerica Group, Inc. of Elwood, Kansas is the licensee of NovaGene, Ltd. and Baylor College of Medicine under U.S. Patent No. 4,514,497. After extensive testing of the vaccine by TechAmerica Group, Inc. under the review of the United States Department of Agriculture (USDA), the USDA issued a product license to TechAmerica Group, Inc., to market the pseudorabies vaccine. The pseudorabies vaccine is marketed under the name "OMNIVAC-PRV".

A second patent, i.e., U.S. Patent No. 4,569,840, issued on February 11, 1986 as a result of our research. This patent covers a novel bovine herpesvirus vaccine and is jointly owned by NovaGene, Ltd. and Baylor College of Medicine.

Patents are pending on second-generation vaccines for both pseudorabies virus and bovine herpesvirus.

2. The Vaccines.

Pseudorabies in swine and bovine herpesvirus in cattle are highly destructive within our nation's livestock industry, resulting in an estimated \$55 million in annual losses. As much as 14% of the 70 million swine in the U.S. is infected with pseudorabies, which causes reduced growth, infertility, abortion and death. Bovine herpesvirus infects about 65% of the cattle in the U.S.

Our vaccine is the first modified-live virus vaccine of its kind. Although all modified live virus vaccines are genetically altered, previous vaccines were developed by a hit-or-miss process, much like a blind dentist attempting to do a root canal. If he drills long enough on enough teeth, he will eventually find the right one, but it will be a long and painful process.

On the other hand, our work was like the dentist who knows the right tooth and the benefit of X-rays and good vision. That is, we knew our target, a gene named thymidine kinase (TK), which when inactivated makes the virus safer, while still protective against pseudorabies disease.

Our vaccine is safer than previous vaccines, not only because it contains a deletion in the TK gene, but also because it cannot revert to an infectious form. In contrast, conventional live virus vaccines can revert to an infectious form. Even if our vaccine did revert, it would revert to a commercially used pseudorabies vaccine since in the development of our vaccine, we employed such as a starting material.

Another advantage of our vaccine is that it is distinguishable, for epidemiological purposes, from field strains of PRV and other vaccine strains of PRV.

3. Testing.

The first tests of our vaccine was done in my laboratory at the Baylor College of Medicine with mice and rabbits under controlled conditions with the full approval of the Institutional Biosafety Committee.

Second phase testing was done by Dr. Eugene Pirtle, Ph.D., the senior scientist in pseudorabies at the USDA's National Animal Disease Center in Ames, Iowa. Dr. Pirtle carried out the first pig safety experiments. In these experiments, he used 100 million infectious units of the vaccine per pig. The vaccine's effectiveness was demonstrated by challenging the treated pigs with more than 500 million infectious units of an illness-causing PRV. None of the pigs developed signs of disease. Normally 10 thousand units of the virulent strain can kill a pig.

Subsequently we were asked to use the vaccine to help stop an outbreak of pseudorabies on a Texas farm. This proposal was discussed for three months with veterinarians at Texas A&M University and Dr. John Holcombe, the Animal Health Commissioner of the State of Texas. Dr. Holcombe approved the experiment to use the vaccine in order to attempt to prevent the spread of the pseudorabies outbreak. The State of Texas and NovaGene, Ltd. jointly financed the test. We have followed the herd for two years and there is no sign of pseudorabies disease on the farm.

We also carried out small pilot experiments on lambs and calves. These experiments demonstrated that the vaccine is safe in lambs and calves and that the vaccine virus is not transmitted from animal to animal. It should be noted that even if the virus had been transmitted, it would not cause illness because the TK gene, i.e., the PRV gene that facilitates replication and re-infection, has been removed from the virus.

4. Recombinant DNA and the Environment.

Our work was done in full compliance with the guidelines of Veterinarian Services No. 868, which states that "in normal husbandry and laboratory practices, veterinary biological products are not considered to be released into the environment."

In January, 1986, the Environmental Protection Agency submitted a report at the Paris meeting of the Organization of Economic Cooperation and Development. The report deals with the safety considerations for commercial and environmental applications of organisms derived by recombinant DNA technology. The report states that "genetic engineering of organisms existed long before the ability to modify directly the cell materials. It dates from man's recognition that animals and crop plants could be selected and crossed to produce the desired phenotype.... Consequently there is no scientific justification for singling out recombinant DNA work for a special regulatory regime over and above that which is already established to regulate traditional biotechnology."

5. Second Generation Vaccines.

Our work on a second-generation virus vaccine was carried out in response to the need to distinguish by a blood test, a vaccinated animal from an infected animal. Our second-generation pseudorabies vaccine can safely and simply distinguish between vaccinated pigs and infected animals.

Conclusion

Our hope is to eliminate pseudorabies and bovine herpesvirus from the farms of the United States and other countries through vaccination programs. It will save the livestock industry many millions of dollars a year. Our nation and the world cannot have healthy people without a healthy food supply. We hope we can help farmers protect the health of the meat supply through the vaccines we have developed.

We may also contribute something to protecting our wildlife endangered species. We have been asked by the University of Florida at Gainesville to collaborate in tests to save the Florida panther, the state animal. There are fewer than 50 still

alive. These panthers feed on wild pigs, 35% of which are infected with pseudorabies virus. We are concerned that pseudorabies virus may be a cause of death of some panthers because pseudorabies virus causes fatal infections in cats. We believe that our vaccine can also be used to help save the endangered Florida panther.

Most of all, we believe that our research and its applications to vaccine development will provide data applicable to the design of human vaccines, and that this work will contribute to the economic well-being of the agricultural industry and to the overall health of the human population.

TESTIMONY

by

Saul Kit, Ph.D.
Head, Division of Biochemical Virology
Baylor College of Medicine
One Baylor Plaza
Houston, Texas 77030

BEFORE THE
HOUSE SUBCOMMITTEE ON INVESTIGATIONS AND OVERSIGHT
OF THE COMMITTEE ON SCIENCE AND TECHNOLOGY

APRIL 29, 1986

TABLE OF CONTENTS

- PART ONE:** What is Pseudorabies and Why is a Vaccine Necessary?
- PART TWO:** Work Done by NOVAGENE, LTD. and the BAYLOR COLLEGE OF MEDICINE in the Development of a Vaccine
- PART THREE:** Description of Techniques for Alteration of the Thymidine Kinase Gene of PRV and How Our PRV Vaccine Differs from Previous PRV Vaccines
- PART FOUR:** Pre-Licensing Safety and Efficacy Tests Conducted by Dr. Saul Kit and Collaborators
- PART FIVE:** Relationships of Dr. Saul Kit with TECHAMERICA GROUP, INC. and with the USDA as it Relates to the USDA Licensing of the Vaccine
- PART SIX:** Guidelines of the National Institutes of Health Recombinant DNA Advisory Committee
- PART SEVEN:** The Second Generation Pseudorabies Virus Vaccine

PART ONE

What is Pseudorabies and Why is a Vaccine Necessary?

What is Pseudorabies and Why is a Vaccine Necessary?

It has been estimated (Animal Health Source Book, 1984 Edition) that there are about 780 million pigs worldwide (294 million in China, 147 million in Eastern Europe and the USSR, 105 million in Western Europe, 94 million in Central and South America, 10 million in Canada, and 50-60 million in the USA). In Taiwan, there are 18 million people and seven million pigs. There are more pigs in the Peoples' Republic of China than people in the United States of America. Swine products are a major source of food; further, swine producers engage in one of the major sectors of economic activity.

Besides domestic pigs, there are at least a million feral swine distributed in 20 of the United States (see: Exhibit A-1). Some feral swine are the descendants of pigs released by the Spanish conquistadors in the seventeenth century to provide a food supply. Other feral swine were imported into Southern states by hunting clubs.

Pseudorabies (also known as "mad itch" and "Aujeszky's disease") is a highly contagious disease of swine and other livestock, such as cattle, sheep, and goats, and is caused by Herpesvirus suis also known as pseudorabies virus or "PRV". In swine, the disease causes respiratory illness and encephalitis, which may progress in 3 to 7 days to death. Other common consequences of infection in swine are abortions, neonatal demise, reduced litter size, and slower growth rates. In other livestock, most notably, calves and lambs, pseudorabies is almost invariably a rapidly fatal disease. Pruritis (or itching) is common prior to death. Other animal species, such as dogs, cats, rodents, raccoons, and fox, can also be fatally infected by PRV, but are considered dead-end hosts; i.e., they are not considered to be sources of infection of other animals. Transmission of pseudorabies commonly occurs from pigs to other pigs, or from pigs to other species. Fortunately, clinical disease has never been shown to occur in humans exposed to the pseudorabies herpesvirus, or to PRV-infected swine, or to pork products.

Aujeszky, a Hungarian physician and veterinarian living in Budapest, is credited with the first description of pseudorabies, which he published in 1902. Aujeszky demonstrated that a disease occurring naturally in cattle, dogs, and cats was transmissible in rabbits, in which it produced characteristic signs which terminated in death. Richard Shope, in 1931, established that "mad itch" occurring in cattle in Iowa was the same as Aujeszky's disease.

Pseudorabies is a major threat and the cause of economic loss to the swine industry worldwide. Within the last 10 years, economic losses have escalated. It has been estimated that about

9% of the hogs on farms in the United States are infected, in comparison to less than 0.8% a decade ago (see: Exhibit A-2). W.J. Mackey, State of Minnesota Board of Animal Health, has estimated that a 100% increase in infected herds occurred in 1981 over 1980. 1982 showed a similar high incidence rate. Two hundred and five infected herds were known in Minnesota, and Mackey calculated that there might be 400 such herds (see: Exhibit A-3). The incidence of pseudorabies is likewise high in the feral pig population. Recent surveys of trapped or hunter-killed wild hogs in California have shown them to be 3% seropositive for PRV. Tests run in Texas in 1984 showed that 18% of the feral Piney Wood rooster hogs were seropositive for PRV. In certain parts of Florida, Dr. E.C. Pirtle, Lead Scientist, Pseudorabies Research, Virological Research Laboratory, National Animal Disease Center, Ames, Iowa, this year found that 35% of the feral pigs were seropositive for PRV. Dr. Victor Nettles, Professor of the University of Georgia, has reported that about 2% of the feral pigs of Ossabaw Island, Georgia, are seropositive for PRV.

The Livestock Conservation Institute of South St. Paul, Minnesota has described several approaches to the elimination of PRV from swine herds. These fall into three broad categories: (i) test and slaughter; (ii) depopulation-repopulation; and (iii) vaccination. Of these, the test and slaughter policies have most often been used, particularly in the United Kingdom. However, they have been costly, difficult to apply successfully in large, continuous farrowing operations, unpopular with producers, and they have not eliminated pseudorabies disease from the United Kingdom. Depopulation-repopulation as a method of pseudorabies eradication is disruptive to the purebred and commercial producers, who may suffer severe economic losses from depopulation, loss of valuable bloodlines, and disrupted cash flow. The vaccines which were available in 1982 were of economic value to the swine producers in that they reduced death losses and the cost of production. However, they did not preclude vaccinated swine becoming infected, developing the carrier state, and spreading the disease. Also, swine vaccinated with the available vaccines confused blood testing procedures because vaccinated swine could not be differentiated serologically from swine infected with deadly field strains (see: Exhibit A-4). Available vaccines had a bad reputation with users who considered them dangerous for pregnant animals and piglets from birth to 10 days of age (see: Exhibit A-5). Accidental vaccine-caused fatalities in lambs have been described in the United States and Europe.

On the basis of 25 years of research on the biochemistry of viruses, it was clearly known to me and to the founders of NOVAGENE, LTD. that the existing vaccines could be substantially improved. The methods for doing so were straightforward and within our capabilities. This knowledge spurred the foundation of NOVAGENE, LTD.

Exhibit A-1

Letter from V.B. Scott, Associate Specialist,
Louisiana Cooperative Extension Service, to
E.C. Pirtle, Ph.D. dated September 12, 1984

Letter from E. Paul J. Gibbs, Professor of Virology,
College of Veterinary Medicine, to Dr. Saul Kit,
dated April 22, 1986

Article Entitled "Pseudorabies and Brucellosis in East
Texas Piney Wood Rooted Hogs" by Bruce Lawhorn, DVM



September 12, 1984

 KNOX Hall
 Baton Rouge, LA 70803-1900
 504 388-4141

E. C. Pirtle, Ph.D.
 USDA Virological Research Laboratory
 P. O. Box 70
 Ames, Iowa 50010

Dear Dr. Pirtle:

This letter is in response to your letter of August 8, inquiring about feral swine in Louisiana. I have contacted all of the people I know who might have knowledge concerning the information you seek. No one could really add anything on the subject to the information I already had access to. However, I did contact the La. Wildlife and Fisheries to confirm the information I am going to pass on to you. The enclosed pamphlet from the La. Wildlife and Fisheries is for your information and may be useful to you in securing additional information on feral swine in Louisiana.

Truly feral swine used to be imported into Louisiana on a regular basis to maintain and add new blood lines for several of the hunting clubs in the State. However, Wildlife and Fisheries personnel believe this practice was discontinued five to ten years ago. It is assumed that most of the wild hogs in Louisiana, today, have migrated from domestic herds and have reverted to the wild state. It is also a fairly accurate assumption that many of the wild hogs in the areas they inhabit have genetic backgrounds linked to the feral imports through matings with descendants of these imports.

There are reported sightings and taking of wild hogs in most all parishes throughout Louisiana. However, the largest numbers are observed in the lowlands and river bottoms encompassed by our large areas of Kisatchie National Forest and Wildlife Management area. The largest numbers of wild hogs are known to exist in the central and southeastern parishes of Louisiana. If you need accurate estimates of numbers and specific locations, I suggest you contact Louisiana Wildlife and Fisheries for this information.

Another item concerning feral swine that may interest you is that the LSU Department of Animal Science has a small herd of feral swine at the LSU Baton Rouge Swine Farm. These pigs originated from the Darien Islands off the coast of Georgia about 15 years ago. They were first used by the University of Georgia research teams and later for some USDA studies at Auburn University. The feral herd was moved to LSU about 1 1/2 years ago. The herd is being used

Page 2
E. C. Pirtle
Sept. 12, 1984

for parasite studies by the LSU College of Veterinary Medicine and the LSU Department of Animal Science. For further information you may want to contact the LSU Department of Animal Science here at the Baton Rouge, Louisiana campus.

I hope this simple overview supplies the basic information you requested on feral swine in Louisiana. If I can be of further assistance, please don't hesitate to contact me.

Sincerely,

V B Scott

V. B. Scott
Associate Specialist
(Animal Science)

VBS/shc

cc: Dr. W. H. Waters
Dr. L. L. McCormick
Dr. Bill Turner
Dr. Lee Southern
Dr. Bonner Stewart



COLLEGE OF VETERINARY MEDICINE
J. HILLIS MILLER HEALTH CENTER
INSTITUTE OF FOOD AND AGRICULTURAL SCIENCES

DEPARTMENT OF INFECTIOUS DISEASES
BOX J-127, JMMHC
UNIVERSITY OF FLORIDA
GAINESVILLE, FLORIDA 32610
PHONE RESPONSE TO:

April 22, 1986

Dr. Saul Kit,
Division of Biochemical Virology,
Baylor College of Medicine
Houston, TX 77030

Dear Saul:

I am writing to ask your advice and collaboration on ways in which we can promote the survival of the endangered Florida panther (*Felis concolor coryi*). As you are probably aware the panther is the state animal for Florida and there are probably fewer than 50 still alive.

There are many possible reasons for the decline of this sub-species in this century. Urban development is probably the most important, but infectious diseases, particularly viral diseases, may also be affecting its survival.

To explain why I am asking your advice, it is necessary to outline a little of the history of Florida. Shortly after the discovery of Florida by the Spanish, they introduced swine to their settlements. Some of these swine were released into the wild and were the progenitors of the large population of feral swine (approximately 500,000) that now exists in the state. We know from serological surveys that pseudorabies virus - or at least a virus very closely related to it - commonly infects this population. Pseudorabies virus generally causes a fatal infection in domestic cats fed meat from infected swine. Since we know from examination of feces that the Florida panther feeds on feral swine, we are concerned that pseudorabies virus may be a cause of death of some panthers. (Let me add, however, that we have no direct evidence that any panthers have died of pseudorabies).

The state has an established research and wildlife conservation program directed to protecting the Florida panther in its native environment. Many of the panthers are closely monitored and vaccination of some individuals to protect them from pseudorabies is a feasible proposal.

This long preamble brings me to the point of this letter. I would be interested to know whether the "genetically engineered" pseudorabies vaccine developed by you and introduced to the veterinary market under the tradename of Omnivac-PRV has ever been tested in cats. A preliminary discussion with Dr. Norman of Tech. America on March 12th led me to believe it has not been tested in cats. In view of the apparent safety of this vaccine in ruminants, it holds considerable promise for use in felidae.

Dr. Saul Kit
April 22, 1986
Page Two

To evaluate the safety of the vaccine, I would be pleased if you would comment on the experimental design outlined below. Since I believe that research in this area will be mutually beneficial, may I suggest that we try and establish a co-operative project.

Experimental protocol to evaluate the safety in felidae of Omnivac-PBC pseudorabies vaccine; a genetically engineered vaccine.

(The protocol is designed to sequentially evaluate the safety of the vaccine in domestic and captive species before possible use in the field).

Phase 1: Evaluation in domestic cats (*Felis catus*)

- a) Vaccinate 15 cats with standard vaccine dose by intramuscular inoculation.
- b) House with 5 non-vaccinated cats serving as controls.
- c) Monitor all cats for clinical disease daily and for virus excretion and development of serum antibody on days 0, 4, 7, 10, 14 and 21 days.
- d) Challenge vaccinated cats, in contact control cats, and a further 3 non-vaccinated cats previously unexposed to the vaccinees with $10^{5.0}$ log 10 tissue culture infected doses of the Indiana Funkhouser strain of pseudorabies virus per cat.
- e) Monitor all cats as in 'c'.

Note: all domestic cats will be specific-pathogen free and not previously vaccinated against feline viral rhinotracheitis.

Phase 2: Evaluate in Bob cats (*Felis rufus*) captured in Florida using same protocol.

Phase 3: Evaluate in captive Mountain lions/Western cougar (*Felis concolor*) using same protocol (Numbers reduced to 6, 2 and 2 respectively).

Phase 4: Evaluate in captive Florida panther(s) (*Felis concolor coryi*) as considered appropriate. (only one available at present).

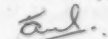
At this time, we are exploring various avenues of financial support for this project. I should also point out that the proposal has not reached the stage where it has been presented to the Animal Care Committee of the University which oversees all animal experiments on campus.

I am sure you will also see that a detailed study of the pathogenesis of pseudorabies virus in the cat could be developed from these studies.

I look forward to your reply.

With best wishes.

Yours sincerely,



E. Paul J. Gibbs
Professor of Virology

EPJB/seb

cc: Dr. E. Pirtle
Mr. T. Hines

PSEUDORABIES AND BRUCELLOSIS IN EAST TEXAS PINEY WOOD ROOTER HOGS

Bruce Lawhorn, DVM

Extension Swine Veterinarian

(1953)
On Saturday, May 26, the Piney Wood Rooter Association of America held its first Texas breeding stock sale in Liberty County. Although piney wood rooster-type hogs are sold weekly through many East Texas auction markets, Ken Horton, executive vice president of the Texas Pork Producers Association, was contacted by several local TPPA members concerning this fast-approaching public sale. Their concern was that these "recently" captured and domesticated wild-type hogs might transmit diseases to domestic swine exposed from such a public sale. As a result, the Texas Animal Health Commission and the Texas Agricultural Extension Service rapidly responded by pre-sale blood sampling of all swine for pseudorabies and brucellosis testing. All sale pigs were also identified by metal ear tag and permitted to the premise of destination in case follow-up testing was necessary.

Results of Initial Testing

After receiving test results on all seventy-six piney wood rooters sampled at the sale, fourteen (18.4%) were positive for pseudorabies (PRV) and eight (10.5%) were positive for brucellosis. A total of eighteen animals were positive and four reacted to both tests. Four of seven consignors having positive hogs were from Walker, Liberty, Polk, and Harris Counties. The three consignors bringing negative hogs were from Trinity and Newton Counties. Including all consignors and buyers at this May 26 sale, sixteen premises were initially subject to testing because of positive and/or exposed hogs. Montgomery County was one additional county involved by purchased hogs.

Follow-up Testing

As of July 12, one hundred twenty-three additional completed tests and/or retests from consignor, buyer, or exposed herds showed twenty additional PRV

and eight additional brucellosis positives. Wild swine tested but not brought to the sale made up nearly all additional positives. Of three animals that tested "clean" at the sale, one was positive for PRV, one was positive for brucellosis, and one was a brucellosis suspect on follow-up testing. Results on one hundred thirty-one exposed domestic swine were pending test completion.

Three PRV positive hogs were transported to the Texas Veterinary Medical Diagnostic Lab in College Station for pseudorabies virus isolation procedures. Results of these studies are not yet concluded. Tissues from three brucellosis positive hogs were submitted to the Austin State-Federal Diagnostic Lab for brucellosis culture and isolation, but were uninterpretable due to contaminants. Tissues from additional brucellosis positives will be submitted for further culture work.

Herd owners have been extremely cooperative during these efforts and four pseudorabies positive hogs have been donated for the aforementioned research.

Sources of Infection

Since domestic swine brucellosis is uncommon in Texas, no previous PRV or brucellosis quarantined domestic swine herds have occurred in East Texas, and clinical PRV disease in other livestock has not been diagnosed in Texas, sources for PRV and brucellosis infection in piney wood rooters remain speculative. Follow-up testing to date suggests that PRV and brucellosis are endemic or exist in a certain percentage of piney wood rooster or wild-type East Texas hogs. Recent disease surveys of trapped or hunter-killed wild swine in other states tend to support this assumption. For example, a 1982 survey of five southeastern states (including Louisiana and Arkansas) showed a six percent positive rate for brucellosis in wild swine. In this study, Brucella suis as the cause of brucellosis was cultured from positive hogs in Louisiana and Florida. In a 1983 disease survey of California wild hogs, a fifteen percent brucellosis positive and a three percent positive PRV rate was found. The

Brucella suis bacteria was also recovered from brucellosis positive hogs in this study.

Compared to these surveys, the higher rate of PRV positives found on the initial sale test may indicate that herds created from wild swine, or breeding stock "borrowing" or "trading" practices increase disease incidence above naturally-occurring endemic levels. The observation that many older piney wood rooster herd boars and mature sows are positive for PRV and/or brucellosis tend to support this theory.

Brucellosis Disease Risks to Humans and Other Animals

Since many piney wood rooster hogs eventually reach the human food chain after trapping, hunting, or individual purchases through auction markets or at private sale, the possible disease transmission risks to humans, domestic pigs, and other animals come into question. Swine brucellosis is termed a zoonotic disease because it is transmissible from swine to man. Acute and chronic disease can occur in man with mortality possible in about two percent of cases. Swine brucellosis can be caused by Brucella suis, Brucella abortus (cattle type), or other Brucella bacteria species. If infection in swine is caused by any species other than Brucella suis, the pig becomes a "dead-end" host, and possible transmission to other animals except humans is thought unimportant.

Human infection may occur through cuts or abrasions following handling infected wild hogs, their viscera, or their meat. Breathing in the bacteria, ingestion by mouth, or contamination of the eyes are other ways of infection. Precautions such as use of disposable rubber gloves and thoroughly washing contaminated skin with an antiseptic or disinfectant soap should be practiced when exposure is possible. Eating the meat of brucellosis positive wild swine is not known to be hazardous following thorough cooking.

Even though domestic swine brucellosis is uncommon, one recent South Central Texas occurrence of brucellosis abortion in domestic pigs was associated with, but not proven to originate from, wild swine.

PRV Disease Risks to Humans and Other Animals

Clinical disease has never been shown to occur in humans exposed to the pseudorabies herpes virus, PRV-infected swine, or pork products. The pig is the natural host for PRV. This means that many pigs survive infection and become possible sources for non-infected swine, other livestock, cats, dogs, and wild animals. In susceptible species except swine, PRV infection is usually fatal. For this reason it is recommended the meat or viscera from possible PRV-infected hogs not be fed or accidentally left exposed for pet, wild animal, or livestock access.

It is tempting to blame recent outbreaks of PRV in domestic swine herds in Central and South Central Texas on associated wild hog populations. At present only one PRV positive wild hog has been found near one of these herds and transmission to this domestic herd has not been proven.

Summary

There is no question that serologic proof of previous PRV and brucellosis infection exists in this diverse group of piney wood rooster hogs tested. At present the actual transmission threat from wild pigs to domestic swine and other livestock is unclear. However, because of PRV and brucellosis and the many other swine diseases these wild-type hogs may carry, producers should attempt to keep domestic swine and other livestock separated from wild-type swine.

This information given herein is for educational purposes only. Reference to commercial products or trade names is made with the understanding that no discrimination is intended and no endorsement by the Cooperative Extension Service is implied.

Educational programs conducted by the Texas Agricultural Extension Service serve people of all ages regardless of socio-economic level, race, color, sex, religion, or national origin.

SELECTED REFERENCES

1. Deyoe BL: Brucellosis. Leman AD, Glock RD, Mengeling WL, et al (ed): Diseases of Swine, ed 5. Ames, Iowa, The Iowa State University Press, 1981, pp 410-417.
2. Gastafson DP: Pseudorabies. Leman AD, Glock RD, Mengeling WL, et al (ed): Diseases of Swine, ed 5. Ames, Iowa, The Iowa State University Press, 1981, pp 410-417.
3. Howard JL, Armstrong JR: B. melitensis. Fact Sheet #L-2064, Texas Agricultural Extension Service, Texas A&M University, College Station, Texas, 1983 Reprint.
- ✓ 4. Zygmunt SM, Nettles VF, Shotts EB, et al: Brucellosis in wild swine: A serologic and bacteriologic survey in the southeastern United States and Hawaii. J Am Vet Med Assoc 181:1285-1287, 1982.
- ✓ 5. Clark RK, Jessup DA, Hird DW, et al: Serologic survey of California wild hogs for antibodies against selected zoonotic disease agents. J Am Vet Med Assoc 183:1248-1251, 1983.
6. Brucellosis Progress Report. Livestock Conservation Institute, 2 (April 1982): 4.

Exhibit A-2

U.S. Government Survey Data

U.S. GOVERNMENT SURVEY DATA
1960-1969
(1960-1969)

Reproduced at U.S. GOVERNMENT Expense

Pseudorabies Slaughter Serum Survey (March 1983-February 1984)



Pseudorabies Slaughter Serum Survey (March 1983-February 1984)

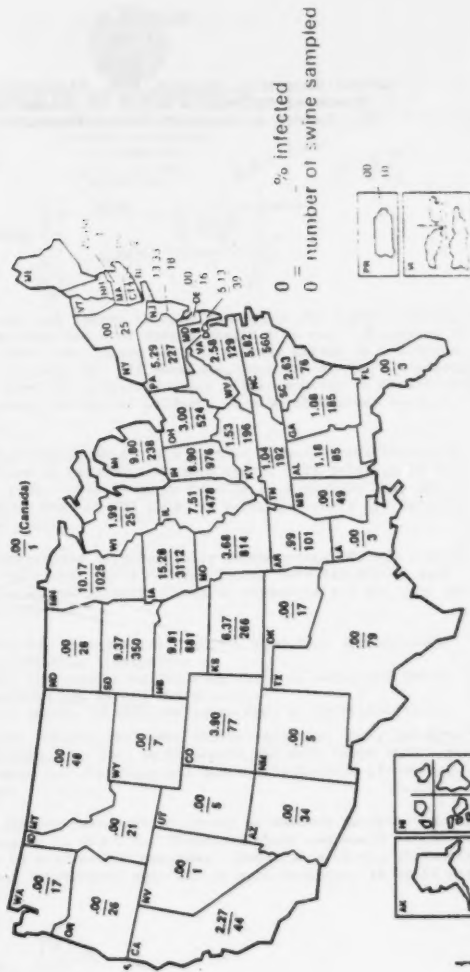


Exhibit A-3

Letter from W.J. Mackey, DVM, Assistant Secretary,
State of Minnesota Board of Animal Health to
Mr. Robert A. Axelrad dated September 21, 1982

September 21, 1982

STATE OF MINNESOTA

BOARD OF ANIMAL HEALTH
 11 70 METRO SQUARE BLDG
 7TH & ROBERT STS.
 ST. PAUL, MN. 55101
 (612) 224-1842

Mr. Robert A. Axelrad
 DYCHE & WRIGHT
 Attorneys at Law
 815 Walker Avenue
 1600 Nellie Esperson Building
 Houston, TX 77002

Dear Sir:

Mr. Mark Seetin has referred your letter to this department for reply. As the Assistant Secretary of the State Board of Animal Health I am very interested in developments which could effect the course of this serious threat to the Minnesota livestock industry, pseudorabies. If NovaGene, Inc. can develop a pseudorabies vaccine which will have the properties they are attempting to attain they will have developed a valuable biological product. I wish them great success in their efforts.

To justify my statement that Pseudorabies is a serious threat to the livestock industry let me say that here in Minnesota we have seen a 100% increase in infected herds in 1981 over 1980. 1982 seems to be showing a similar high incidence rate. To be specific we know of 203 infected herds and there may well be many others we do not know about.

The economic impact of pseudorabies is potentially devastating. We did a study of 14 infected herds and found that their average losses were \$23,500.00 each (multiply that by an estimated 400 infected herds in Minnesota and you have approximate losses near \$10 million).

It is well agreed that existing Pseudorabies vaccines have some serious deficiencies from a disease eradication standpoint:

- (a) They do not prevent the carrier or shedding state in swine and therefore vaccinated animals can spread the disease.
- (b) A vaccination titer cannot be differentiated from an infection titer.

As a result of these vaccine deficiencies many states recommend their use only in infected or in high risk herds. We here in Minnesota may soon adopt such a policy. In defence of vaccines I must say that they are quite effective in preventing abortions and death losses.

Today's vaccines have not stopped Pseudorabies spread in eastern European countries and do not seem to be doing so in the U.S. Therefore their use would be counter productive in any control or eradication programs. Vaccines with the characteristics you listed would be a great improvement over our current vaccines, if it is possible to develop them.

Sincerely,

W. J. Mackey, DVM

W. J. Mackey, D.V.M.
 Assistant Secretary

WJM:jp

cc: Mr. Mark Seetin

AN EQUAL OPPORTUNITY EMPLOYER



Letter from Harry C. Mussman,
Administrator of Animal and Plant Health Inspection
Service, USDA, to Mr. Robert A. Axelrad dated September 9, 1982



United States
Department of
Agriculture

Animal and
Plant Health
Inspection Service

Washington, DC
20250

September 9, 1982

Mr. Robert A. Axelrad
Dyche and Wright
815 Walker Avenue
1600 Mellie Esperson Building
Houston, TX 77002

Dear Mr. Axelrad:

Enclosed is a copy of the Economic Analysis of Pseudorabies Program Options prepared by this Service, March 26, 1981, which contains answers to most of the questions in your letter of August 24, 1982.

A vaccine with the properties outlined in your letter would indeed be a valuable tool in the control and eradication of pseudorabies. Vaccines available on the market at this time are of economic value to the swine producer in that they reduce death losses and cost of production. However, they do not preclude vaccinated swine becoming infected, developing the carrier state, and transmitting the disease. Also, swine vaccinated with present vaccines cannot be serologically differentiated from infected swine.

We hope this material meets your needs.

Sincerely,

Harry C. Mussman
Administrator

Enclosure

Exhibit A-5

Letter from William H. Beckenhauer, DVM, Vice President,
Research and Development, Norden Laboratories, to
Mr. Robert A. Axelrad dated September 10, 1982

NORDEN

September 10, 1982

Mr. Robert A. Axelrad
 Dyche and Wright
 815 Walker Avenue
 1600 Nellie Esperson Building
 Houston, TX 77002

Dear Mr. Axelrad:

Thank you for your letter of August 24 on novel vaccines for herpesviruses to be developed by NovaGene, Inc.

We are definitely interested in new, better herpesvirus vaccines. We "pioneered" the first pseudorabies vaccine licensed in the U.S. and presently market herpesvirus vaccines for cattle (IBR), horses (rhinopneumonitis), cats (rhinotracheitis) and pigs (pseudorabies).

The properties you described for the new vaccine are close to ideal. There is some reluctance developing in the customer base to use of live virus vaccines. Probably based more on theoretical rather than actual safety concerns, but it is a consideration to keep in mind for NovaGene managers. I would particularly urge rigorous safety studies in pregnant animals (pseudorabies has a bad reputation in these) and in pigs from birth to 10 days of age, because pseudorabies mortality approaches 100% in piglets of this age.

Norden Laboratories is receptive to discussing a business arrangement with NovaGene to manufacture and market novel vaccines. We are willing to meet with you to discuss the disease, vaccine business, product development and regulatory considerations at your convenience.

Again, thanks for contacting Norden.

Sincerely yours,



William H. Beckenhauer, D.V.M.
 Vice President, Research and Development

WMB:er

of a Vaccine

Work Done by NOVAGENE, LTD. and the BAYLOR COLLEGE OF MEDICINE
in the Development of a Vaccine

NOVAGENE, INC., a Texas Corporation, was organized in the latter half of 1982 by Robert A. Axelrad, Esq., of Houston, Texas, and my son, Dr. Malon Kit, M.D. Dr. Malon Kit is the President and Robert A. Axelrad, Esq. is the Executive Vice President of NOVAGENE, INC. Market analyses and feasibility studies were conducted at the time the company was founded and the need for a pseudorabies vaccine was established through inquiries to the United States Department of Agriculture, State Animal Health officials, and numerous veterinary manufacturing/marketing companies (see: Exhibit B-1). All of the research funds for the development of these novel, genetically engineered pseudorabies vaccine were raised by Robert Axelrad, Esq. and Dr. Malon Kit from Venture Capital through a Limited Partnership offering (NOVAGENE, LTD.).

Research on the new vaccine was conducted in the Division of Biochemical Virology laboratories of the BAYLOR COLLEGE OF MEDICINE through a contract which provided that patent rights would be jointly assigned to NOVAGENE, LTD. and the BAYLOR COLLEGE OF MEDICINE. I was named as the Principal Investigator to direct the research. Dr. Malon Kit had a major role in carrying out the laboratory experiments. I also served as a scientific spokesman for the project and, during 1983 and 1984, explained ongoing results and the significance of the research to several veterinary manufacturing companies, the general public, and to other scientists. Dr. Stewart McConnell, Professor of the Department of Veterinary Microbiology and Parasitology, Texas A&M University, was enlisted as a Scientific Consultant for NOVAGENE, LTD. Professor McConnell retired from Texas A&M in July of 1985 and was named Professor Emeritus. He now serves as a consultant for the USDA. He recently accepted a position as Vice President of Animal Health Research at NOVAGENE, INC.

Research on the pseudorabies vaccine began January 1, 1983 and two candidate vaccines were developed. U.S. Patent No. 4,514,497 has issued on these novel vaccines.

The first two vaccines developed by NOVAGENE, LTD. and the BAYLOR COLLEGE OF MEDICINE was a mutagen-induced, temperature-resistant, thymidine kinase-negative Bucharest strain of pseudorabies virus, designated PRV(BUK-SA). The second vaccine was a temperature-resistant, thymidine kinase-negative deletion mutant of the Bucharest strain of pseudorabies virus, designated PRV(BUK-dl 3). PRV(BUK-dl 3) has been licensed by the USDA as OMNIVAC-PRV. Recently, a second generation vaccine, designated PRV(dlg92/dltk), has been developed and was described

at the First Annual Biotechnology Meeting of the American Society for Microbiology held in Washington, D.C., March 20-23, 1986 (see: Exhibit B-2).

NOVAGENE, LTD. has also developed a temperature-resistant, mutagen-induced, thymidine kinase-negative infectious bovine rhinotracheitis (bovine herpesvirus-1) vaccine. U.S. Patent No. 4,569,840 was issued on this novel vaccine. NOVAGENE, LTD. has also developed a thymidine kinase deletion mutant of infectious bovine rhinotracheitis virus, but has not yet field tested it (see: Exhibit B-3).

Exhibit B-1

Letter from Robert A. Axelrad, Esq., Dyche & Wright, to
Beecham Laboratories dated December 9, 1982

Letter from Robert A. Axelrad, Esq., to
Jensen-Salsbery Laboratories, dated December 9, 1982

Letter from Robert A. Axelrad, Esq., Dyche & Wright, to
Dellen Laboratories, Inc., dated December 9, 1982

Letter from Robert A. Axelrad, Esq., Dyche & Wright, to
Pitman-Moore, Inc., dated December 9, 1982

DYCHE & WRIGHT
ATTORNEYS AT LAW
818 WALKER AVENUE
1800 MELLIE ESPERSON BUILDING
HOUSTON, TEXAS 77002

TELEPHONE (713) 223-1418
CABLE: DYCHWRIGHT
TELEX: 702184
TELECOPIER 224-3824
(DIRECT LINE AFTER HOURS)

ROBERT A. AXELRAD

December 9, 1982

Beecham Laboratories
Vice President/Marketing
501 Fifth Street
Bristol, Tennessee 37620

Dear Sir:

This firm represents NovaGene, Inc., a newly formed genetic engineering corporation in the state of Texas. NovaGene, Inc. is preparing to embark on an R&D program to develop a new generation of vaccines against herpesvirus diseases. We are very optimistic about the prospects for the development of vaccines with the following properties:

- 1) a modified live virus- for rapidity, strength, and extended duration of protection
- 2) capable of preventing the carrier state
- 3) with associated methods to differentiate between animals carrying the disease and those which have been immunized.

It is our understanding that a pseudorabies vaccine with these unique features would be invaluable in future efforts to control and eradicate this disease.

A critical assumption in our business plan is that such a pseudorabies vaccine, if developed, could be licensed to a pharmaceutical corporation with existing manufacturing and marketing capabilities, and an appreciation of the economic potential of a pseudorabies vaccine with the described features.

We are writing to you at this time in order to inquire whether you could envision the establishment of this type of relationship, and whether an interest might exist in the described improvements in vaccine technology.

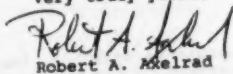
Your willingness to express a potential interest will be pivotal in justifying investment in this promising area.

Page 2
Beecham Laboratories
December 9, 1982

If you should have any interest in the above project, please
write to the undersigned attorney.

Thank you for your kind consideration.

Very truly yours,


Robert A. Adelrad

RAA/pl

DYCHE & WRIGHT

ATTORNEYS AT LAW
815 WALKER AVENUE
1600 MELLIE ESPERSON BUILDING
HOUSTON, TEXAS 77002

TELEPHONE (713) 823-1415
CABLE: DYCHWRIGHT
TELEX: 792184
TELECOPIER 824-3824
(DIRECT LINE AFTER HOURS)

ROBERT A. AXELRAD

December 9, 1982

Jensen-Salsbery Laboratories
Vice President/Marketing
P. O. Box 167
Kansas City, Missouri 64141

Dear Sir:

This firm represents NovaGene, Inc., a newly formed genetic engineering corporation in the state of Texas. NovaGene, Inc. is preparing to embark on an R&D program to develop a new generation of vaccines against herpesvirus diseases. We are very optimistic about the prospects for the development of vaccines with the following properties:

- 1) a modified live virus- for rapidity, strength, and extended duration of protection
- 2) capable of preventing the carrier state
- 3) with associated methods to differentiate between animals carrying the disease and those which have been immunized.

It is our understanding that a pseudorabies vaccine with these unique features would be invaluable in future efforts to control and eradicate this disease.

A critical assumption in our business plan is that such a pseudorabies vaccine, if developed, could be licensed to a pharmaceutical corporation with existing manufacturing and marketing capabilities, and an appreciation of the economic potential of a pseudorabies vaccine with the described features.

We are writing to you at this time in order to inquire whether you could envision the establishment of this type of relationship, and whether an interest might exist in the described improvements in vaccine technology.

Your willingness to express a potential interest will be pivotal in justifying investment in this promising area.

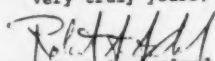
-2-

Page 2
Jensen-salsbery Laboratories
December 9, 1982

If you should have any interest in the above project, please
write to the undersigned attorney.

Thank you for your kind consideration.

Very truly yours,


Robert A. Axelrad

RAA/pl

DYCHE & WRIGHT
ATTORNEYS AT LAW
818 WALKER AVENUE
600 MELLIE ESPERSON BUILDING
HOUSTON, TEXAS 77002

TELEPHONE (713) 223-1415
CABLE: DYCHWRIGHT
TELEX: 792184
TELECOPIER 224-3824
10:00 PM LINE AFTER HOURS

ROBERT A. ARELRAO

December 9, 1982

Dellen Laboratories, Inc.
Vice President/Marketing
8445 Lake Street
Omaha, Nebraska 68134

Dear Sir:

This firm represents NovaGene, Inc., a newly formed genetic engineering corporation in the state of Texas. NovaGene, Inc. is preparing to embark on an R&D program to develop a new generation of vaccines against herpesvirus diseases. We are very optimistic about the prospects for the development of vaccines with the following properties:

- 1) a modified live virus- for rapidity, strength, and extended duration of protection
- 2) capable of preventing the carrier state
- 3) with associated methods to differentiate between animals carrying the disease and those which have been immunized.

It is our understanding that a pseudorabies vaccine with these unique features would be invaluable in future efforts to control and eradicate this disease.

A critical assumption in our business plan is that such a pseudorabies vaccine, if developed, could be licensed to a pharmaceutical corporation with existing manufacturing and marketing capabilities, and an appreciation of the economic potential of a pseudorabies vaccine with the described features.

We are writing to you at this time in order to inquire whether you could envision the establishment of this type of relationship, and whether an interest might exist in the described improvements in vaccine technology.

Your willingness to express a potential interest will be pivotal in justifying investment in this promising area.

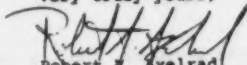
-2-

Dellen Laboratories, Inc.
Page 2
December 9, 1982

If you should have any interest in the above project, please
write to the undersigned attorney.

Thank you for your kind consideration.

Very truly yours,


Robert A. Axelrad

RAA/pl

DYCHE & WRIGHT

ATTORNEYS AT LAW
818 WALKER AVENUE
1800 MELLIE ESPERSON BUILDING
HOUSTON, TEXAS 77002

TELEPHONE (713) 223-1418
CABLE: DYCHWRIGHT
TELEX: 708184
TELECOPIER 224-3824
(D-RECEIVE LINE AFTER HOURS)

ROBERT A. AXELRAD

December 9, 1982

Pitman-Moore, Inc.
Vice President/Marketing
P. O. Box 344
Washington Crossing, N.J. 08560

Dear Sir:

This firm represents NovaGene, Inc., a newly formed genetic engineering corporation in the state of Texas. NovaGene, Inc. is preparing to embark on an R&D program to develop a new generation of vaccines against herpesvirus diseases. We are very optimistic about the prospects for the development of vaccines with the following properties:

- 1) a modified live virus- for rapidity, strength, and extended duration of protection
- 2) capable of preventing the carrier state
- 3) with associated methods to differentiate between animals carrying the disease and those which have been immunized.

It is our understanding that a pseudorabies vaccine with these unique features would be invaluable in future efforts to control and eradicate this disease.

A critical assumption in our business plan is that such a pseudorabies vaccine, if developed, could be licensed to a pharmaceutical corporation with existing manufacturing and marketing capabilities, and an appreciation of the economic potential of a pseudorabies vaccine with the described features.

We are writing to you at this time in order to inquire whether you could envision the establishment of this type of relationship, and whether an interest might exist in the described improvements in vaccine technology.

Your willingness to express a potential interest will be pivotal in justifying investment in this promising area.

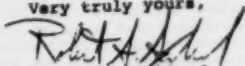
-2-

Pate 2
Pitman-Moore, Inc.
December 9, 1982

If you should have any interest in the above project, please
write to the undersigned attorney.

Thank you for your kind consideration.

Very truly yours,


Robert A. Akelrad

RAA/pl

Exhibit B-2

Abstract for the First Annual ASM Conference on Biotechnology
20-23 March 1986, Washington, D.C.

First Annual ASM Conference on Biotechnology

20-23 March 1986 Washington, D.C.

Official Abstract Form

Instructions: Complete this form and submit it, with all required enclosures, for receipt by 31 January 1986. Only this original form is acceptable (no photocopies). Additional forms are available from the ASM Conferences Department. Refer to the sample abstract on p. vi for style. Type the title (initial capitals only) first; then list all authors (all capital letters), with an asterisk for the person delivering the paper; and then list institutions and short addresses (do not give departments, divisions, buildings, etc.). Note: Any poorly prepared abstract unsuitable for direct reproduction will be retyped, and the author will be charged \$20.

ABSTRACT

Pigs Vaccinated with a Genetically Engineered PRV Deletion Mutant Are Distinguishable Serologically from Pigs with Natural PRV Infections. S. KIT,* M. SHEPPARD, and M. KIT. Baylor College of Medicine, Houston, TX.

Test and slaughter programs have been unsuccessful in controlling pseudorabies (PR), a highly contagious and often fatal disease of swine and other livestock caused by a herpesvirus (PRV). Current vaccines also are inadequate because: (i) there is no test that differentiates vaccinated pigs from naturally infected pigs; (ii) federal regulations presume that all seropositive pigs are latently infected and forbid interstate movement to markets. A safe vaccine that protects livestock from PR, but does not produce a positive PRV test, is needed. Recently, a thymidine kinase deletion mutant, PRV(BUK-d13), was shown to be safe and efficacious as a vaccine in pregnant pigs, piglets, calves, and sheep. Second generation vaccine, PRV(dlgCdlk), was engineered to have a deletion in a PRV glycoprotein, gC. ³H-mannose-labeled extracts from wt PRV-infected cells, which contain gC, were immunoprecipitated with PRV antisera from: (i) hyperimmunized pigs; (ii) naturally infected domestic or feral swine; and (iii) pigs vaccinated with PRV(dlgCdlk). SDS-PAGE and autoradiography showed that sera (i)-(ii) reacted with wt gC, but sera from (iii) did not. Labeled extracts from PRV(dlgCdlk)-infected cells did not contain gC. Thus, for the first time, vaccinated pigs may be distinguished from naturally infected pigs.

Complete the following:

☒ This material will not have been published or presented at a scientific meeting before 20 March 1986.

Full name and mailing address of presenting author

Saul Kit, Ph. D.

Head, Division of Biochemical Virology

BAYLOR COLLEGE OF MEDICINE

One Baylor Plaza

Houston,

TX

77030

Telephone (713) 799-4507

City

State

Zip

vii

Susannah, submitted Jan 28, 1986 - not yet accepted.
 Confirmed. Do not disclose prior to March 20, 1986.

Exhibit B-3

Veterinary Record dated March 15, 1986

was at the same time removed from contiguous areas.

The following illustrates the recovery rate

Year	Number of Cases	Number alive on culture
1982	14	8
1983	14	8
1984	18	7
1985	18	13

All three cases were confirmed and showed microscopic lesions following histological examination.

The reason for the improved recovery rate in 1985 is unclear. The use of antibiotic for treatment did not appear to influence our results, nor the age of the animal submitted. The common serotypes of *L. monocytogenes* 1/2 and 4 were most commonly recovered over this period with type 3 on one occasion only.

While it is possible that cold enrichment techniques could have improved our recovery rate, the time and effort involved in pursuing the matter in a diagnostic laboratory might hardly justify the return. The limited number of bovine cases recorded during the same period suggested this technique might give similar or slightly poorer recovery rates.

N. S. M. MACLEOD
R. WELLS
Veterinary Investigation Centre
West of Scotland Agricultural
College
Dumfries

Reference
LOW, C. & LINELLER, R. (1985) *Veterinary Record*
Supplement to Practice, March, 308

Control of Aujeszky's disease

From Dr S. Kit and others

SIR, — We have read with interest the letters by J. T. van Oirschot (*VR*, November 16, 1985, p553) and D. Todd and J. B. McFerran (*VR*, December 14, 1985, p647) concerning the possibilities for new vaccination/eradication programmes of Aujeszky's disease, based upon vaccines which permit veterinarians to discriminate between vaccinated pigs and pigs naturally infected with Aujeszky's disease virus.

Recently, we described a thymidine kinase (TK) deletion mutant derived from the Bucharest strain of Aujeszky's disease virus (Kit and others 1985). This genetically engineered modified live virus vaccine, Omni-vac-PRV (Tech America) has been successfully used in a vaccination/eradication programme to eliminate Aujeszky's disease from a farrow to finish operation in west central Texas. Sows in all stages of pregnancy, newborn piglets, non-pregnant females and boars were vaccinated in this study.

In addition, we have engineered a second generation Aujeszky's disease virus, designated (PRV)(dgC/dtk), which contains a new deletion in a gene encoding a major envelope glycoprotein. Despite deletion mutations, PRV(dgC/dtk) replicates to titres exceeding 10^7 pfu/ml in cultures of

swine testicle cells. Pigs immunised with PRV(dgC/dtk) are protected from disease after challenge exposure to over 10^6 pfu of the highly virulent Indiana-Funkhouser strain of Aujeszky's disease virus, and develop antibodies to the disulphide-linked (gH) glycoprotein complex, but not to the deleted major glycoprotein. Furthermore, Aujeszky's disease virus antisera from pigs hyperimmunised with virulent Aujeszky's disease virus strains and from naturally infected domestic and feral pigs immunoprecipitate the major glycoprotein from extracts of virulent Aujeszky's disease virus infected cells, but not from extracts of PRV(dgC/dtk)-infected cells. Thus, it is now possible to: (i) distinguish by simple and cheap serological tests pigs vaccinated with our second generation virus from pigs naturally infected with Aujeszky's disease virus or vaccinated with those virus strains which express the major Aujeszky's disease virus glycoprotein; and (ii) control Aujeszky's disease through vaccination/eradication programmes.

SAUL KIT
MICHAEL SHEPPARD
Baylor College of Medicine
Houston
MALON KIT
NovaGene, Inc
Houston
Texas 77032
USA

Reference
KIT, S., KIT, M. & PIRTLE, E. C. (1985) *American Journal of Veterinary Research* 46, 1336

Uterine prolapse in the cow

From Professor C. Forniston

SIR, — When I was a pupil in a Cheshire practice 60 years ago prolapse of the bovine uterus was commonplace. These were the days before epidural anaesthesia and in consequence any method or positioning of the patient which assisted the reduction of the prolapsed organ was a bonus. It was therefore with some interest and revival of memories that I read the contribution to *In Practice* by Bob Plenderleith (January, p14). I can give him a 'practice tip' which will enable him to position his cow as depicted in his article much more easily. It is a method used by the late William Kendrick of Tarrun, a man who taught me the art and science of country practice as no other man could.

Assuming the patient is standing in its box, a bale of straw or truss of hay is placed under its belly just in front of its udder. The nose end of a rope is placed about the pasture of the left foreleg and the rope is passed over the cow's neck to the right side. When pulled the cow buckles on to its knees and its hind legs automatically shoot backwards. During this simple manipulation the cow's head should be controlled and turned laterally if necessary.

The cow in this position needs no further restraint and is a 'pruner' until the bale or truss is removed. To support the prolapse, in those days a mangle board was more rigid and more effective than a sheet and was likely to be more 'sterile'.

In the process of kneading back the uterus

The Veterinary Record, March 16, 1986

I was taught to tuck one's thumbs well into clenched fists, to avoid puncture of the uterus particularly if it was turgid. Once reduced, involution could be encouraged by the introduction of water at body temperature to be syphoned off almost immediately. Intrauterine medication and the application of a West's clamp to the vulva completed the exercise. In retrospect a West's clamp appeared to me to be somewhat vicious. Labial sutures were often used, they were less drastic and just as effective.

C. FORNISTON
4 Marlow Court
Chase Side
London N14

From Mr J. Douch

SIR, — I read Mr R. W. Johnston's letter (*VR*, March 1, p252) with interest. The method of dealing with uterine prolapse which he describes is exactly the one I was taught 30 years ago in the West Country.

May a small animal practitioner add two points? One, in a difficult case, the sprinkling of two pounds or so of sugar on the everted organ works wonders in reducing the oedema. Secondly, take great care in selecting the point to which you attach the block and tackle: the first time I tried the method I came within an ace of pulling the cowhead down about my ears.

JOHN DOUCH
33 Castle Street
Dover

From Mr C. D. Parker

SIR, — I was shocked by Mr Johnston's letter (*VR*, March 1, p252) in which he describes his method for replacing uterine prolapse in the cow.

Many cows with 'posterior paralysis' are uncomfortable and distressed by their condition. Therefore to intentionally produce such a state, using an epidural, without any form of sedation, 'to put the patient down' is at the least inconsiderate. Subsequently to hurt the animal's hind end three feet by its hocks is, in my opinion, inhumane.

How can we educate the farming community and general public about animal welfare, when some members of our profession continue to advocate such archaic methods?

C. D. PARKER
Allyn Saxon Drive
Shepton Mallet
Somerset

Gastric dilation in dogs

From Mr R. Jantz

SIR, — I was very interested to read the recent letters concerning the gastric dilation-torsion complex (*VR*, January 25, p110; February 15, p190).

Although none of the patients I have treated for this condition have been bloodhounds, I have found a dramatic increase in the incidence of this condition recently. During 1985 I treated 19 cases surgically. All these cases were in the 1st breeds such as the Irish wolfhound, the

PART THREE

Description of Techniques for Alteration of the Thymidine
Kinase Gene of PRV and
How Our PRV Vaccine Differs from Previous PRV Vaccines

Description of Techniques for Alteration of the Thymidine

Kinase Gene of PRV and

How Our PRV Vaccine Differs from Previous PRV Vaccines

Our vaccine [PRV(BUK-dl 3); OMNIVAC-PRV] is the first tailor-made, designer vaccine to have been developed and brought to market. Like all other modified-live virus (MLV) vaccines, OMNIVAC-PRV is a genetically altered virus. To explain the difference between OMNIVAC-PRV and all previous MLV vaccines, I will briefly describe how previous vaccines were developed and how our vaccine was developed (see: Exhibit C-1).

Most previous vaccines have been prepared by a random, hit or miss process. The starting material used to prepare our vaccine was a cloned substrain of the Bucharest strain of PRV and is an example of such previous vaccines. Other substrains of the Bucharest PRV strain have been used as vaccines for over 25 years in Europe and for at least 8 years in the U.S.A.

Specifically, the Bucharest strain of PRV was attenuated by repeated (400 to 800) passages of the virus in chick cells, a foreign host. This technique is an adaptation of the one originally used by Pasteur to obtain a rabies vaccine. Genetic alterations accumulate during repeated passages of viruses in foreign hosts. It's analogous to the famous Irish play by Lady Gregory in which a gossip tale is told and retold. Errors accumulate at each retelling of the story so that at the tenth repetition the story is totally different from the original one.

Veterinarians had no idea until 1984 as to how many genetic alterations had occurred in the Bucharest strain, where in the virus genome they occurred, at which passage they occurred, or the functional significance of any of the genetic alterations. However, with the advent of restriction nuclease fingerprinting analyses, it became apparent to us that the most important genetic alteration that had occurred in the Bucharest strain was a deletion mutation of the BamHI-12 fragment of PRV DNA (see: Exhibit C-2). It is noteworthy that this same deletion of the BamHI-12 fragment is found in two other widely used PRV vaccine strains, Bartha (Hungary) and NIA-4 (Ireland). By using the "marker transfer" techniques of modern molecular biology, the BamHI-12 fragment of PRV DNA can be restored to the vaccine strains. The resulting reconstituted virus is pathogenic. In contrast, after deliberate genetic alterations of pathogenic PRV strains so as to delete the BamHI-12 fragment, apathogenic vaccine-like viruses are produced.

It has been shown that the BamHI-12 fragment of PRV DNA encodes a PRV envelope glycoprotein, named gI. This gI glycoprotein probably facilitates the entry/egress relative to infectivity of PRV from host cells and/or the spread of the virus from the site of infection to the central nervous system. Viruses lacking the gI gene may be impaired in their ability to carry out these functions. The gI gene which produces the gI glycoprotein is located in the unique short (U_S) region of PRV DNA.

In the 1960's, a second general approach to the development of virus vaccines was introduced. An example is the infectious bovine rhinotracheitis virus vaccine (IBR), named "Tracherine", and manufactured by Smith-Kline Animal Health, Ltd. The IBR vaccine virus was deliberately altered by treating virus-infected cells with a conventionally used potent chemical mutagen, i.e., nitrous acid. From the many genetically altered (mutated) IBRV progeny, temperature-sensitive viruses were selected. This was carried out by "plaque-purifying" the viruses (standard virological methods) and selecting a virus clone that grows well at a low temperature (33°C), but not at a higher temperature (39°C or 40°C). The developers of temperature-sensitive virus vaccines did not know how many virus genes had been altered by nitrous acid treatment, their location in the virus DNA, or the specific function of the altered virus genes. It is known, however, that all such temperature-sensitive (conditional lethal) mutations alter virus genes that are critical for the duplication of infectious particles in tissue culture.

The temperature-sensitive IBR vaccine cannot be administered subcutaneously or intramuscularly; it must be given intranasally (a very inconvenient route of administration). The theory is that the vaccine virus will replicate at the comparatively low temperature of the nasal mucosa and elicit an immune response, but that the vaccine virus will not be able to replicate in the lungs or deep in the body tissues, where the temperature will be higher (38.5°C - 40°C). Thus, virus replication will be self-limiting.

Genetically altered, "cold-sensitive" human influenza virus vaccines which have been developed are analogous in principal to the heat-sensitive IBR vaccine described above.

The PRV vaccine virus discussed above designated PRV(BUK-5A) is a mutagen (bromodeoxyuridine (BrdUrd))-induced thymidine kinase-negative (TK⁻), heat-resistant PRV vaccine virus which can replicate efficiently over the broad temperature range of 32°C to 40°C . A thymidine kinase negative virus is one which fails to produce the enzyme thymidine kinase. Production of this enzyme is necessary for establishing latency in the host. This vaccine virus was derived by a deliberate genetic alteration of the

pseudorabies TK gene using conventional chemical mutagenesis technology. Thus, PRV(BUK-5A) vaccine, was not developed through recombinant DNA technology but, rather, conventional chemical mutagenesis technology.

We discovered that the TK gene is located at one end of the BamHI-11 fragment of PRV DNA in the unique long (U_L) region of PRV DNA (see: Exhibit C-3).

PRV(BUK-dl 3) (OMNIVAC-PRV) is a temperature resistant TK⁻ deletion mutant of the Bucharest strain of pseudorabies virus. PRV(BUK-dl 3) was developed by deleting the PRV TK gene from the Bucharest strain of pseudorabies virus using the marker transfer technique. The procedure involved the addition, to millions of living animal (rabbits) cells in tissue culture, of a mixture of intact, infectious DNA from the parent TK⁺ PRV strain plus a modified BamHI-11 fragment of PRV DNA which contained a genetically engineered deletion in the TK gene. After the two components of PRV DNA entered the cells, the infectious parental TK⁺ PRV DNA was copied many times by the cell and infectious virus particles (viral DNA enclosed in a protein coat) were produced. In a few of the virus progeny, a portion of the TK⁺ PRV DNA was exchanged by "homologous recombination" for the modified BamHI-11 PRV DNA fragment containing a deletion in the TK gene. As a result, these viral progeny were TK⁻ deletion mutants of PRV. Homologous recombination is a naturally occurring event in cells which goes on all of the time. We took advantage of the process and "encouraged" the infected cell to generate the types of genetically altered viruses that we wanted (in this case, a virus with a deletion in the TK gene). The next task, which we successfully accomplished, was to select from the millions of virus particles, the few PRV which were TK⁻ deletion mutants.

Now, why is OMNIVAC-PRV superior to MLV TK⁺ PRV vaccines?

(1) OMNIVAC-PRV is a deletion mutant. Thus, it cannot revert (change back) to the parental-type TK⁺ virus.

The possibility that virus will revert to the pathogenic form is a constant fear with previously developed vaccines. For example, Kew and coworkers reported in the J. Gen. Virol. 56, 337-347, 1981 that:

Immunization with live virus oral polio vaccines is highly effective in preventing poliomyelitis. The oral polio vaccine strains currently used were selected as spontaneous mutants of wild isolates following numerous passages in simian extraneural cells and tissues.

Despite the many advantages of using attenuated viruses to produce conditions resembling natural infections, a general concern with live virus vaccines is the potential of the viruses to revert to virulence upon replication in humans. Opportunities for reversion appear to be clearly present because the oral polio vaccine strains are probably multi-site mutants of the revertible missense class, and several lines of evidence indicate that the vaccines can undergo genetic variation upon human intestinal passage. Indeed, in developed countries, a significant fraction of the few remaining annual cases of paralytic poliomyelitis occur among vaccinees and their contacts. The problem of vaccine-associated disease arises most frequently with the vaccines of serotypes 2 and 3. (Literature citations are omitted.)

It should be noted that vaccine-associated poliomyelitis also occurs after administration of killed vaccines, as in the case of the famous Cutter incident.

Let me emphasize that even if OMNIVAC-PRV did revert genetically, we would be back to the original Bucharest virus, which has been employed as a vaccine strain.

(2) OMNIVAC-PRV can be distinguished from all other field strains or vaccine strains of PRV.

OMNIVAC-PRV can be distinguished from all other field strains or vaccine strains of PRV by comparing the biochemical properties, and also the DNA thereof with the corresponding biochemical properties and DNA of any other strains of PRV. This capability is important for epidemiological purposes. Should vaccinated pigs ever develop pseudorabies, we can examine pseudorabies virus isolates from the sick animals and determine with 100% accuracy whether the animals were infected with a field virus strain, our vaccine virus, or both.

(3) OMNIVAC-PRV is the first virus vaccine brought to market that was genetically altered by the most up-to-date, state of the art methods with a specific target so that deliberate genetic alterations which we made would increase safety without loss of efficacy. The target, the thymidine kinase gene, was chosen because we now have a large body of scientific evidence on herpesviruses showing that TK mutants: (i) are less pathogenic than their TK parents (i.e., less likely to produce clinical illness); (ii) do not replicate as well as TK parents in non-dividing nerve cells; (iii) are highly unlikely to be

reactivated from latency in sensory ganglia; and (iv) protect animals against clinical disease caused by virulent herpesvirus strains.

OMNIVAC-PRV administered intramuscularly is not shed intranasally from vaccinated pigs. Even if it were shed and transmitted to another pig, that animal would be vaccinated and not harmed. My colleagues in Texas vaccinated about 200 pregnant sows in all stages of pregnancy. The size of litters was normal, the piglets did not develop any clinical signs of disease, and none of these pigs developed PRV antibodies, showing that their mothers did not transmit the virus to them.

(4) OMNIVAC-PRV is safe.

The increased margin of safety of OMNIVAC-PRV has been demonstrated experimentally. The results of these experiments have been published in Kit *et al.*, *Am. J. Vet. Res.* 46, 1359-1365 (1985).

(5) OMNIVAC-PRV is user friendly.

OMNIVAC-PRV fits easily into producers' management schemes, is inexpensive and is given only once and without adjuvant.

Exhibit C-1

"Vaccine", by Malon Kit and Saul Kit in McGraw-Hill
Yearbook of Science and Technology, 1986, New York:
McGraw-Hill Book Company, pp. 478-480

In: *McGraw-Hill Yearbook of Science and Technology*. 1986.
New York: McGraw-Hill Book Company.

478 Vaccine

Fig. 3a and b are closely related (by a Fourier transform) as both describe the acoustic wavefield in the vicinity of a fold caustic. The differences in appearance of the two is due to the different nature of the associated acoustic-wave-generating mechanisms.

Thus, the catastrophes appear naturally in underwater acoustics in two related contexts. The caustics of geometric acoustics take the form of the catastrophes as do the time histories of the acoustic wavefields in the vicinity of the caustics.

For background information see REFRACTION OF WAVES; UNDERWATER SOUND in the McGraw-Hill Encyclopedia of Science and Technology.

[MICHAEL G. BROWN]

Bibliography: M. V. Berry and C. Uspall, Catastrophe optics: Morphologies of caustics and their diffraction patterns, *Prog. Opt.*, 18:257-346, 1980; Allan D. Pierce, *Acoustics: An Introduction to Its Physical Principles and Applications*, 1981.

Vaccine

The prevention of viral diseases by active immunization with inactivated or attenuated viruses has been critical to the control of such maladies as smallpox, rabies, yellow fever, and poliomyelitis. Unfortunately, many viral diseases remain beyond the reach of conventional vaccine technology. Further progress is predicated upon a thorough understanding of the molecular basis of antigenicity and virulence. However, the recent application of recombinant deoxyribonucleic acid (DNA) technology, computer simulation, and biopolymer sequencing and synthesis now permit the detailed analysis of viral structure, function, and host interactions. From these studies is emerging a new generation of vaccines, which are described below, using herpes simplex virus as an example.

Conventional vaccines. Conventional "killed" vaccines consist of virus particles whose infectivity has been destroyed with chemicals. These inactivated virus particles retain the ability to elicit immune responses and to protect the host from infection. The protective immunity that arises subsequent to vaccination results from host reactions to the virus surface proteins, which often represent only a fraction of all the proteins present in the virus particles. These host reactions include the synthesis of several classes of antibodies, proliferation and functional activation of various kinds of lymphoid cells and macrophages, and the production by these cells of interferons and other lymphokines.

It appears that there are distinct though overlapping groups of viral antigens responsible for stimulating antibodies and cell-mediated immunity. Neutralization of virus infectivity occurs when restricted molecular domains on the proteins in the external shell or surface of virus particles, called antigenic determinants or epitopes, are bound by antibodies. Neutralizing antibodies may interfere with absorption of a virus to the cell, or may induce conformational changes which interfere with virus penetration and uncoating. Antibodies induced by viral antigens

may also bind to viral proteins on the surface of infected cells, thereby eliciting cytotoxicity, which may be antibody-dependent, cell-mediated or antibody-dependent, complement-mediated.

Structural analysis of antigenic determinants of viral proteins has shown that they are often hydrophilic, solvent-accessible regions where loops and turns in the polypeptide backbone occur. Furthermore, antigenic determinants correspond to those polypeptide segments with greater degrees of conformational flexibility. Defining at a molecular level those components of viral particles that elicit reactive antibodies may permit the design of vaccines free from residual surviving virus or other irrelevant components which may interfere with safety or efficacy.

Herpes simplex virus. Herpes simplex is a medium-sized virus, 150-200 nanometers in diameter, with one double-stranded DNA molecule of about 150 kilobases (kb) that is enclosed in a protective protein shell (capsid). The capsid together with the DNA, or nucleocapsid, is about 100 nm in diameter and is surrounded by a lipid-containing envelope. The viral envelope includes four major glycoproteins, designated gB, gC, gD, and gE, which induce neutralizing antibodies and cell-mediated immune responses.

Herpes simplex virus causes oral, ocular, genital, and neuronal infections. The incidence of genital herpes infections has been increasing in recent years. Studies of the sexual transmission of genital herpes virus reveal that many persons who transmit the disease are either asymptomatic or have unrecognized symptoms at the time of transmission. Since antiviral drugs cannot eradicate established latent herpes virus infections, herpes virus vaccines have a potential role in disease prevention. Concerns regarding the potential oncogenicity of herpes virus DNA, and the evidence that humoral and cell-mediated immune responses are directed largely against the virus glycoproteins, have prompted the development of glycoprotein subunit vaccines free of DNA as the first approach to prevention.

Subunit vaccine. One type of herpes virus subunit vaccine has been derived by stripping the viral envelope from the other constituents by detergent extraction. The combined virus envelope glycoproteins have been further purified by affinity chromatography and, when administered to human volunteers, elicited neutralizing antibodies and cell-mediated immunity. There appear, however, to be qualitative as well as quantitative differences in the immunity induced with the various glycoproteins, and a truly effective vaccine for as complex a virus as herpes simplex may require an experimentally tested balance of the individual envelope glycoproteins.

Considerable attention has been directed at characterizing the structure of one herpes virus glycoprotein, namely, glycoprotein D (gD), since both oral (type 1) and genital (type 2) strains of herpes virus have substantial antigenic cross-reactivity with this

protein compared to the others. Purified gD's from either type 1 or 2 herpes virus stimulate high titers of type-common virus neutralizing antibodies that mediate cytotoxicity. Glycoprotein D (molecular weight 59,000 daltons) also plays a central role in absorption of the virus to the cell. The gene encoding gD has been mapped on the herpes virus DNA, and the entire DNA sequence has been determined.

Synthetic vaccine. Utilizing the universal genetic code, the amino acid sequences of the types 1 and 2 herpes virus gD polypeptides have been predicted from the DNA sequences. It appears that the gD polypeptides consist of 393 amino acids and that each polypeptide contains three glycosylation sites. By using a combination of proteolysis, computer predictions, synthetic peptides, and panels of type-common and type-specific monoclonal antibodies, major antibody binding sites (epitopes) have been determined. Amino acid residues 8-23 define one such major epitope. Computer model predictions of other antigenic determinants based on hydrophilicity and secondary structure as well as the use of synthetic peptides and antiserum reactions has also identified residues 268-287 and 340-356 as conformational epitopes. Thus, peptides containing amino acid residues 8-23, 268-287, and 340-356 coupled to protein carriers are potential vaccines.

Synthetic polypeptides. The use of synthetic polypeptides as vaccines has been advocated because of their stability, purity, and low production costs. In addition, synthetic peptides may elicit neutralizing antibodies to antigenically conserved regions which are not, when part of the full viral protein, capable of stimulating antibodies. This could permit immunization against a wide variety of viral serotypes. Studies are currently under way to ascertain the level of protective immunity produced by synthetic herpes virus polypeptide vaccines. Analogous experiments with synthetic polypeptide vaccines for hepatitis B, foot-and-mouth disease, and influenza have demonstrated the potential usefulness and pitfalls of this approach.

Chimeric DNA. Cloning of the herpes virus gD gene in bacterial plasmids has facilitated the construction of chimeric DNA, which when placed under the control of high-efficiency gene expression systems results in the production of large amounts of gD, either as the authentic protein or as fusion of truncated forms. Recombinant DNA-derived gD produced in *Escherichia coli* has been found to be poorly immunogenic, probably due to incorrect protein folding occurring in the absence of a eukaryotic cell membrane. However, when a plasmid vector, pgD-DHFR, was used for transfer to the gD gene to Chinese hamster ovary cells, abundant quantities of an immunogenic form of gD were obtained. The vector pgD-DHFR consisted of *E. coli* plasmid pBR322 with inserted herpes virus gD and mouse dihydrofolate reductase genes under the control of simian virus 40 early promoters. In selective media containing aminopterin, the herpes virus gD and the mouse dihydrofolate reductase genes were stably cotrans-

ferred in an amplified form. The Chinese hamster ovary cells were sensitive to aminopterin toxicity under ordinary tissue culture conditions, but became aminopterin-resistant, provided that the mouse DHFR gene of the plasmid was expressed. Immunization with this animal cell-derived gD protected mice from lethal challenge with herpes virus.

Hybrid vaccines. Hybrid vaccines have also been constructed in which the gD gene of herpes virus is inserted into the genome of vaccinia, the smallpox vaccine virus. Glycoprotein D is produced in the host during the reproductive cycle of vaccinia by tricking the virus to recognize the herpes virus gene as its own. Vaccination has been shown to result in the production of neutralizing antibodies, and a protective immunity against subsequent exposure to herpes virus occurs. Also, because of the large size of vaccinia, up to a dozen other foreign genes might be incorporated into a single vaccine capable of providing protection to a wide variety of infectious diseases.

Modified live virus vaccine. Attenuated or modified-live virus vaccines are crippled or nonpathogenic viruses derived from virulent field strains which impart immunological cross-reactivity. Modified-live virus vaccines are generally regarded as superior to killed or subunit vaccines because they elicit more complete and balanced immunological responses. Conventional attenuated vaccines have been obtained by repeated passage of virus in cells of a species different than the natural host. During multiple passages in tissue culture cells, mutations accumulate as the virus adapts to its new environment. These mutations adversely affect viral reproduction in the natural host, resulting in lessened virulence. Unfortunately, this process may sometimes be reversed, so that vaccine virus may revert to a virulent state. Furthermore, attenuated virus vaccines may be unsafe in young, atopic, or immunocompromised individuals.

With regard to a specific modified-live virus vaccine for genital herpes, the additional concern exists that genital herpes infection increases the risk of cervical carcinoma. Besides numerous epidemiological studies correlating cervical carcinoma with herpes virus infection, tissue culture studies have demonstrated that herpes virus DNA and fragments of this DNA can transform rodent cells to neoplasia. Attempts to show that particular viral gene products are associated with transformed cells or tumors have failed to identify any tumor-specific viral proteins, as has been the case with adenoviruses and papovaviruses. These findings have led to speculation that the oncogenicity of herpes virus may operate through novel mechanisms. The "hit-and-run" hypothesis suggests that the virus alters the cell to a neoplastic or preneoplastic state that does not require the continued presence of the complete virus for its maintenance. An 0.8-kb fragment of herpes virus DNA (about 1% of the total DNA) can substitute for the transforming ability of intact viral DNA, and may function as an enhancer-insertion element

which activates cellular oncogenes.

The suspected oncogenic potential of herpes virus does not necessarily preclude modified-live viruses from being used as herpes virus vaccines for three reasons. First, currently available genetic engineering techniques permit the selective excision of identifiable transforming DNA sequences from herpes virus, fulfilling a basic safety requirement. Second, a recent prospective study involving 10,000 women in Czechoslovakia has failed to confirm the direct association between genital herpes infection and cervical cancer. Third, the oncogenic risk due to vaccination has to be balanced against the oncogenic risk from natural infection with virulent herpes virus.

Introduced nonvirulence. A goal of contemporary research is to identify at the molecular level the herpes virus genes responsible for virulence, broadly defined as the ability to cause disease. As with DNA sequences associated with oncogenic potential, virulence genes can be prevented from causing disease by surgically introducing nonreversible deletion mutations into the respective viral genes.

Virulence is a complex phenomenon that varies with each virus agent. For herpes virus, virulent manifestations of infection are ulcerative skin lesions, urinary retention, central nervous system disturbances such as hindleg paralysis in experimental animals, latent nerve cell infections, encephalitis, abortion, generalized infections in neonates and immunocompromised hosts, and cancer.

Virulence for herpes virus has been identified with several genetic loci, which fall into two broad categories: (1) genes determining products essential for herpes virus replication in tissue culture and in living animals; and (2) genes dispensable under ordinary conditions of virus growth in tissue culture but required for the life cycle of the virus in the living host. The first category of genes may be inappropriate for genetic engineering manipulations because their inactivation represents too drastic an attenuation of the virus. That the second class of genes provides a more promising target is evident from recent results utilizing animal models. One of these genes is the thymidine kinase gene. Replication of herpes virus in nondividing cell populations, such as nerve tissue, is dependent upon the virus bringing with it the metabolic machinery necessary for virus growth. The replication of viral DNA is dependent upon precursor pools of nucleotides formed by viral thymidine kinase. The gene for this enzyme has been characterized in detail. Attenuated herpes virus strains, constructed by genetic engineering techniques which delete portions of the thymidine kinase gene, fail to express enzyme activity and are markedly impaired in their ability to grow in nerve cell. These thymidine kinase-negative herpes virus strains less frequently establish ganglionic infections, either acute or latent, and are markedly less likely to ascend into the central nervous system to cause encephalitis. Vaccinated experimental animals develop mild skin lesions, but are solidly pro-

tected from lethal doses of virulent virus.

A second herpes virus gene dispensable for ordinary tissue culture growth but important for latency is $\alpha 22$ which encodes a protein that functions at the very early onset of viral replication. Although mutants lacking this gene replicate in tissue culture, they are defective in establishing latency. Vaccination by $\alpha 22$ mutants has likewise protected laboratory animals against lethal challenge by virulent virus.

Conclusion. It is important to emphasize that the herpes virus vaccines now being developed through genetic engineering techniques appear capable of decreasing the severity of initial infections and decreasing the likelihood of the establishment of recurrent infections. It is doubtful, however, that any of the various types of subunit vaccines can prevent infection of a vaccinated subject by virulent strains of herpes virus and, thus, the continued spread of virulent virus in the population. It is also unlikely that modified-live herpes virus vaccines can totally prevent reinfection of vaccinated subjects and the establishment of latency, but they have the greatest potential for doing so. Given the perplexing and paradoxical nature of herpes infections, the most rational approach appears to be continuation of efforts to develop modified-live virus vaccines that are acceptably safe.

For background information see ANIMAL VIRUS; CELLULAR IMMUNOLOGY; IMMUNITY; VACCINATION; VIRUS in the McGraw-Hill Encyclopedia of Science and Technology. [MALON KIT; SAUL KIT]

Bibliography: R. M. Chanock and R. A. Lerner (eds.), *Modern Approaches to Vaccines: Molecular and Chemical Basis of Virus Virulence and Immunogenicity*, 1984; L. A. Lasky et al., Protection of mice from lethal herpes simplex virus infection by vaccination with a secreted form of cloned glycoprotein D, *Biotechnology*, pp. 527-532, June 1984; G. J. Mertz et al., Herpes simplex virus type-2 glycoprotein-subunit vaccine: Tolerance and humoral and cellular responses in humans, *J. Infect. Dis.*, 150:242-249, 1984; E. Paoletti et al., Construction of live vaccines using genetically engineered poxviruses: Biological activity of vaccinia virus recombinants expressing the hepatitis B virus surface antigen and the herpes simplex virus glycoprotein D, *Proc. Nat. Acad. Sci. USA*, 81:193-197, 1984.

Vibration

In the analysis of vibrations, statistics may be used to study the temporal behavior of the response or to describe the system itself. The first topic falls in the realm of random vibration; the second is variously termed statistical energy analysis, power flow methods, or asymptotic analysis. Of course, there are situations in which descriptions of both the time behavior and the system parameters are statistical.

Many structures that vibrate are thought of as highly deterministic in their mechanical properties such as wall thicknesses, overall size, component weights, and rigidities. When these systems are ex-

Exhibit C-2

Physical map of PRV



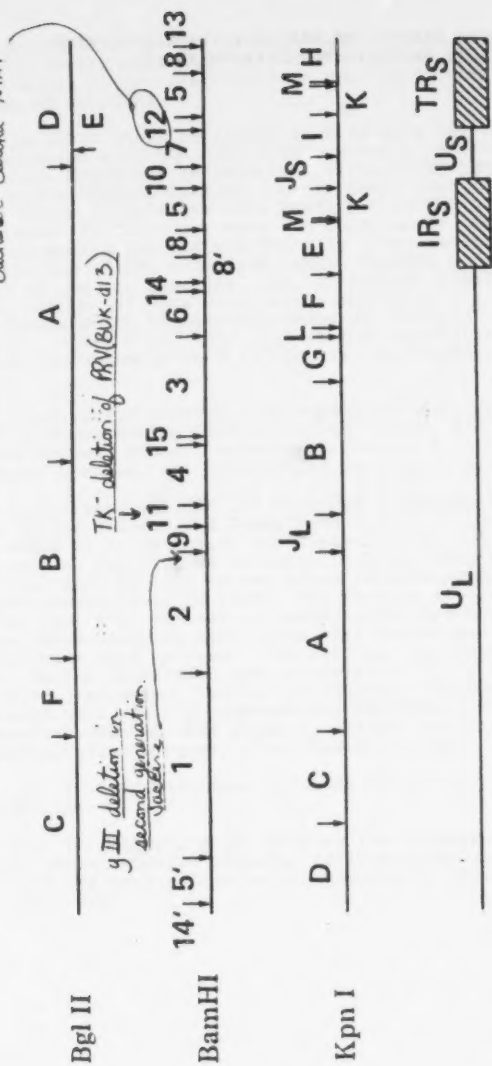


Figure 1

PART FOUR

Pre-Licensing Safety and Efficacy Tests Conducted
by Dr. Saul Kit and Collaborators

Pre-Licensing Safety and Efficacy Tests Conducted
by Dr. Saul Kit and Collaborators

OMNIVAC-PRV was initially tested in mice and rabbits at the Vivarium facilities of the BAYLOR COLLEGE OF MEDICINE with prior notification of the Institutional Biosafety Committees of BAYLOR COLLEGE OF MEDICINE.

The first tests performed in pigs were carried out in collaboration with the USDA's Dr. Eugene C. Pirtle, Ph.D., Lead Scientist, Pseudorabies Research, Virological Research Laboratory, National Animal Disease Center, Ames, Iowa (see: Exhibit D-1). These experiments were performed in CDC Class 3 containment facilities - only one containment level lower than that designated for maximum security. The results of this research and of the mouse and rabbit studies were published in Kit et al., *Am. J. Vet. Res.*, 46, 1359-1369 (1985). Preliminary communications of this work were reported at the 13th Annual UCLA-Symposium on Herpesvirus, held at Steamboat Springs, Colorado, April 8-13, 1984, and at the Ninth International Herpesvirus Workshop held in Seattle, Washington, August 24-29, 1984.

Pilot safety studies were conducted under controlled conditions in Texas during 1984 in calves, lambs, and 3- to 5-day-old piglets by Drs. Stewart McConnell, Charles W. Livingston, Jr., and Bruce Lawhorn, all experienced veterinary virologists.

In February of 1984, an outbreak of pseudorabies occurred in swine on the Wallace and Tommy Maddox farm in Lometa, Texas. We were asked to do detective work (restriction nuclease fingerprinting) on the PRV DNA isolated from the fatally infected baby pigs that were brought to the Texas Veterinary Medicine Diagnostic Laboratory from the farm. The results of our test helped to confirm that the outbreak of pseudorabies had been caused by a pseudorabies-carrying feral boar that had broken into the farm and infected sows in heat. The farm was quarantined by the Texas Animal Health Commission and slaughter of all 1,500 animals on this farm considered. As an alternative, the possibility was suggested that our new experimental PRV(BUK-dl 3) vaccine (now licensed as OMNIVAC-PRV) might be useful for an immunization/eradication program. The objectives were:

- (1) To vaccinate once in order to contain the infection by vaccination;
- (2) To identify pigs carrying the disease-causing PRV through the anamnestic response following vaccination, and to cull from the herd all animals suspected of carrying the pathogenic PRV; and

(3) To replace all female pigs used in the breeding operation with "sentinel" females that were born from vaccinated mothers but never themselves vaccinated.

Meanwhile serology tests were performed on this quarantined swine herd by the Texas Animal Health Commission. Finally, after numerous discussions and with the cooperation of the Maddox family, the Texas Pork Producers' Association, the Texas Animal Health Commission, the Texas Agricultural Extension Service, and NOVAGENE, LTD., we submitted an experimental vaccination/eradication plan drafted by Drs. Stewart McConnell and Bruce Lawhorn. This plan was approved by Dr. John W. Holcombe, D.V.M., Executive Director, Texas Animal Health Commission, Austin, Texas (see: Exhibit D-2).

We supplied sufficient PRV(BUK-dl 3) for vaccination to Dr. Bruce Lawhorn and, finally, on June 26 and 27, 1984, all pigs on this quarantined farm were vaccinated. The 30-day test results were reported to Dr. Holcombe by Dr. Bruce Lawhorn (see: Exhibit D-3). Dr. Lawhorn also later described the results at several veterinary meetings (see: Exhibit D-4).

The results were also reported by me at a Seattle herpesvirus meeting (August 24-29, 1984) and at the 1984 High Technology Route to Virus Vaccine Symposium held in Houston (November 8-10, 1984).

All of this work to assess the safety and efficacy of the vaccine was done and reported prior to the application by TECHAMERICA GROUP, INC. in December of 1984, for a USDA product license.

Exhibit D-1

Letter from Eugene C. Pirtle, Ph.D.,
Research Leader, Pseudorabies, United States
Department of Agriculture dated September 27, 1983
to Dr. Saul Kit

Letter from E.C. Pirtle, Ph.D.
to Robert A. Axelrad dated October 27, 1983

letter from E.C. Pirtle, Ph.D. to Dr. Saul Kit
dated March 5, 1984

Letter from E.C. Pirtle, Ph.D.
to Dr. Saul Kit dated April 13, 1984

Letter from E.C. Pirtle, Ph.D. to Dr. Saul Kit
dated April 17, 1986



United States
Department of
Agriculture

Agricultural
Research
Service

North Central Region
National Animal
Disease Center

P.O. Box 70
Ames, Iowa
50010

Dayton Road

September 27, 1983

Dr. Saul Kit
Head, Division of Biochemical Virology
Baylor College of Medicine
Houston, Texas 77030

Dear Dr. Kit:

I just called our animal supply people to ask about current prices of 5- to 7-week-old pigs. They are \$50.00 each.

Here is what I propose. If you will send me your attenuated pseudorabies virus vaccine (with an infectivity titer for cell culture of at least 10^5 TCID₅₀/ml), I will give 3 pigs one dose of 1.0 ml each and 3 pigs two doses of 1.0 ml each 2 weeks apart. The 6 vaccinated pigs and 2 nonvaccinated controls will be challenged 2 weeks after the last vaccine administration. I will, therefore, need approximately 15 ml of vaccine for pigs and titration in cell culture.

In return for the vaccination trial, I ask to be coauthor on any publication resulting from the vaccine work.

If you wish to discuss this proposal, please call me at (515) 232-0250, ext. 324.

With kindest regards.

Sincerely,

Eugene C. Pirtle
EUGENE C. PIRTLE, Ph.D.
Research Leader, Pseudorabies

Michael P. Davis (Zenkhouse)
10⁵ TCID₅₀ *LD₅₀ mice*
= 80

4.7 x 10⁸ pfu/ml
1.25 ml
1.5 ml
3 pigs (1.5 ml)

25 ml *Jan 9, 1983*

Vaccination 14
Challenge 4.3 x 10⁸ TCID₅₀ PAV (ind)
LD

attention: Dr Michael Wathen
Ed Gibson



United States
Department of
Agriculture

Agricultural
Research
Service

North Central Region
National Animal
Disease Center

P.O. Box 70
Ames, Iowa
50010

OCTOBER 27, 1983

ROBERT A. AXELRAD
VICE PRESIDENT
NOVA GENE, LTD.
PO BOX 270275

DEAR MR. AXELRAD:

I AM RESPONDING TO YOUR LETTER OF OCTOBER 18, 1983.

IN A LETTER TO DR. SAUL KIT (SEPTEMBER 27, 1983), I PROPOSED NOW I WOULD TEST THE ATTENUATED PSEUDORABIES VACCINE THAT HE WOULD PROVIDE. IN TESTING THE VACCINE, I WOULD HAVE COMPLETE CUSTODY OF THE VACCINE, WOULD MAKE IT AVAILABLE TO NO OTHER LOCATION, WOULD DESTROY UNUSED VACCINE AT THE END OF THE VACCINE TRIAL, AND FINALLY, I WOULD SEND RESULTS OF THE VACCINE TRIAL DIRECTLY TO DR. SAUL KIT.

A SATISFACTORY VACCINE TRIAL WITH AN EXPERIMENTAL PSEUDORABIES VACCINE IN COLLABORATION WITH DR. SAUL KIT IN NO WAY EARNS AN ENDORSEMENT OF THE UNITED STATES DEPARTMENT OF AGRICULTURE FOR ANY ENSUING COMMERCIAL PRODUCT.

I BELIEVE THAT THE ABOVE SHOULD SATISFY YOUR PARTICULAR INTERESTS IN THIS MATTER.

SINCERELY,

E. C. Pirtle

E. C. PIRTLE, PhD
RESEARCH LEADER, PSEUDORABIES

CC: DR. SAUL KIT



United States
Department of
Agriculture

Agricultural
Research
Service

North Central Region
National Animal
Disease Center

P.O. Box 70
Ames, Iowa
50010

March 5, 1984

Dr. Saul Kit
Division of Biochemical Virology
Baylor College of Medicine
Houston, TX 77030

Dear Saul:

I am enclosing the procedure and results used for testing the vaccine in pigs. As indicated, the results of virus neutralization tests will be sent to you later--in about two weeks.

If you have any questions regarding the enclosure, please call me.

With kindest regards.

Sincerely,

E. C. Pirtle

E. C. PIRTLE, Ph.D.
Research Leader, Pseudorabies
Virological Research Laboratory

Enclosure



United States
Department of
Agriculture

Agricultural
Research
Service

North Central Region
National Animal
Disease Center

P.O. Box 70
Ames, Iowa
50010

April 13, 1984

Dr. Saul Kit, Head
Division of Biochemical Virology
Baylor College of Medicine
Houston, TX 77030

Dear Saul:

The abstract for the Workshop in Seattle is straightforward, and I suggested only one slight change in the last sentence.

I concur with the amplification of the vaccine study. In addition to the two suggestions you listed, I would add one other. It would be of interest to determine whether or not and for how long the tk⁻ vaccine strain can be recovered from the upper respiratory track after intranasal installation of the virus.

Because of limited space, it will be at least a month before I can do the amplification experiments, but they will be done.

With kindest regards.

Sincerely,

E. C. PIRTLE, Ph.D.
Research Leader, Pseudorabies
Virological Research Laboratory

Enclosure

pages tightly bound



United States
Department of
Agriculture

Agricultural
Research
Service

Central Plains Area
National Animal
Disease Center

P.O. Box 70
Ames, Iowa
50010

April 17, 1986

Dr. Saul Kit
Head, Biochemical Virology
Baylor College of Medicine
Houston, TX 77030

Dear Saul:

At the time I tested the efficacy of the thymidine kinase negative (tk⁻) pseudorabies vaccine in swine, there was no Institutional Biosafety Committee (IBC) at the National Animal Disease Center (NADC) dealing with recombinant DNA situations. As a matter of fact, an IBC was only recently appointed by the Center Director. The local IBC is so new that I would not know of its existence if I had not inquired. It thus appears that we at the NADC will have assistance with recombinant DNA guidelines.

Our animal isolation facilities are Class 3 containment units -- only one class from maximum security. When I tested the tk⁻ pseudorabies vaccine in swine, it was tested in a Class 3 containment unit.

If I can assist with additional information, please contact me.

With kind regards,

Sincerely,

S. C. FITTLE, Ph.D.
Lead Scientist, Pseudorabies Research
Virological Research Laboratory

Exhibit D-2

Letter from Bruce Lawhorn, DVM,
Extension Swine Veterinarian, Texas Agricultural Extension
Service, to John W. Holcombe, DVM, dated May 10, 1984

Letter from John W. Holcombe, DVM, Executive Director,
Texas Animal Health Commission, to Saul Kit, Ph.D.,
dated June 5, 1984

K. 7



**Texas
Agricultural
Extension
Service**

The College of Arts & Sciences
 College Street 77043

Veterinary Medical Administration Building
May 10, 1984 State Capital Complex

May 10, 1984

Scott Reynolds
512-475-4111
Pres. Arnold Health
Commission

John W. Holcombe, DVM
Executive Director
Texas Animal Health Commission
Sam Houston State Office Building
Post Office Box 12966
Austin, Texas 78711-2966

Dear Dr. Holcombe:

Enclosed is a proposed pseudorabies ~~herd eradication~~ ^{eradication} plan for the Wallace and Tommy Maddox swine herd near Longsta. This plan incorporates ~~interim vaccination with an experimental~~ ^{interim vaccination with an experimental} pseudorabies virus developed in the laboratory of Dr. Saul K. Department of Biochemical Virology, Baylor College of Medicine, Houston, Texas. Dr. ~~Edward McDonald's~~ ^{Edward McDonald's} ~~herd plan~~ ^{herd plan} for use of this new vaccine and previous herd eradication plans by Dr. Scott L. Reynolds, I have been combined in this plan. Dr. Thurman L. Fancher has been extensively involved in its development and the herd owners are receptive to its implementation.

Thank you for your time.

Sincerely,

Seine Fankung III

Bruce Lowhorn, DVM
Extension Swine Veterinarian

6/11/20
(407) 845-113

Home
775-7801

Enclosure

cc: Dr. George Shepley - *Head Boy's Board Member*
 Dr. Charles Boyd - *Charles Boyd*
 Dr. Ian Tizard - *Chairman, As. on - College of Science*
 Dr. Stewart McConnell - *Stewart McConnell*
 Scott Reynolds - *Scott Reynolds*
 Thurman Frazier - *Thurman Frazier*
 Dr. James Armstrong - *James Armstrong*
 Dr. Buddy Farley - *Buddy Farley*

The Texas A&M University System, U.S. Department of Agriculture, and the County Commissioners Courts of Texas. www.tamugrass.org

PSEUDORABIES VACCINATION EXPERIMENT FOR WALLACE AND TOMMY MADDOX HERD

The purpose of this vaccination experiment is to eliminate pseudorabies from this herd in the most practical and economical way possible with the least loss of livestock. A live experimental pseudorabies vaccine will be used to attempt blockage of natural infection spread from infected to noninfected animals within the herd or from possibly infected feral swine to noninfected herd swine. The use of this vaccine is necessary because the current test and removal herd plan has not successfully contained virus spreading. In order to attempt pseudorabies elimination from this herd, the following steps will be taken:

1. Experimental Vaccination

- (a) All pigs on the premise on Day 0 regardless of age, sex, or other criteria will be vaccinated IM with a single 2 ml pseudorabies (PRV) virus (BHK-d1 3B) containing 10^7 pfu (pfu = one virus particle).
- (b) Sows that farrow within three to four weeks post vaccination (through Day 30) will be devoid or have reduced PRV maternal antibody to protect piglets from natural infection. Vaccine virus is not thought to spread from vaccinated females to piglets protected or unprotected by maternal PRV antibody. Therefore, all possibly unprotected piglets farrowed through Day 30 should be vaccinated as soon as possible after birth.
- (c) Any piglets through Day 90 that do not receive adequate maternal PRV antibody, as indicated by becoming poor-doer pigs may be totally unprotected from WT PRV infection and possibly spread the virus if infected. These poor-doer pigs should be immediately vaccinated or killed and disposed of by burning or deepburial.
- (d) Anticipated requirements for this study are 1500 doses of vaccine (10^7 pfu) to be given as a single 2 ml dose IM. The Maddox herd contains approximately 175 breeding animals (sows, gilts, and boars) and 1200 growing and finishing pigs at any one point in time. The farrowing and finishing areas are separated, but some breeding and gestation lots are in close proximity to finishing lots. Animal flow is one-way from the farrowing area to finishing except for replacement herd gilts which are returned to breeding and gestation lots. All boars are obtained from off the premises.
- (e) All vaccinated animals are to be marked with a hole punched in the left ear of not less than $\frac{1}{4}$ " in diameter at the time of vaccination. This hole punch instrument will be provided by the Texas Animal Health Commission. All swine in this vaccinated herd will be quarantined to this premise and may be moved only to slaughter.

(f) Product description-pseudorabies virus with the virulent genetic code severed by enzymatic excision. This virus has been developed in the laboratory of Dr. Saul Kit, Department of Biochemical Virology, Baylor College of Medicine, Houston, Texas. Preliminary testing in pigs and calves has shown this virus isolate to be safe and immunogenic. At the present, only limited quantities of this virus are available and no commercial production is anticipated in the immediate future.

(g) Product liability disclaimer-see Attachment 1.

2. Initial Serology

- (a) On Day 0 (day of PRV vaccination) all breeding swine (boars, sows, and gilts including newly selected finishing lot replacements) will be blood sampled for PRV antibody. Individually cleaned and disinfected syringes and needles will be used to sample each animal for testing. The portion of each restraint snare contacting an animal during bleeding will be disinfected before snaring another pig. Footwear will be disinfected before entering each separate lot of swine.
- (b) Personnel of the Texas Animal Health Commission (TAHC) will draw blood for testing. All testing will be done at the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) in College Station or Amarillo and all tests will be done by the serum virus neutralization test (SN test). Testing expense will be borne by the TAHC.
- (c) All Day 0 PRV antibody positive swine will be moved to an isolated reactor lot and shipped to market as soon as possible. Such positive swine are considered latently infected with wild type (WT) PRV and may spread this virus especially if stressed.
- (d) All Day 0 PRV antibody negative swine will be identified as noninfected vaccinates. They will be considered as nonlatent noninfected WT PRV swine pending further testing.

3. Post-Vaccination Serology

- (a) On Days 30, 60, and 90 and any additional approximate 30 day intervals thereafter, post-vaccination breeding herd replacement blood samples will be taken.
- (b) All tested pigs with PRV SN titers of greater than or equal to 1:16 will be considered as suspicious. These animals will be moved to an isolated reactor lot and shipped to market as soon as possible.
- (c) All tested pigs with PRV SN titers of less than or equal to 1:8 will be considered as non-infected vaccinates and be retained within the herd.

- (d) All vaccinated gilts that are selected for breeding stock must have PRV SN titers of 1:8 or less and must not have exhibited PRV signs. This procedure may require additional testing other than on Days 30, 60, 90, etc.
- (e) Prior to introduction into the main herd, all new boars (or any new breeding stock) must be isolated from other swine on the premise for at least 30 days (60 days is better). They also must be tested negative for PRV at least once toward the end of this isolation period.

4. Sentinal Animals

Pigs born to vaccinated sows after Day 30 should be protected by vaccine-induced maternal PRV antibody (provided they nurse properly). These pigs will not be vaccinated and will serve as sentinal pigs after maternal immunity wears off by twelve to eighteen weeks of age.

5. Possible Follow-Up Studies

- (a) Virus study-collect tonsils and trigeminal nerve (right gasserian ganglia) for viral DNA probes to determine latency and type of virus colonizing the tissues.
 - I. Collect tissues for virus isolation from pigs that are aborted and/or die on the premise.
 - II. Collect tissues from selected WT PRV and PRV-dl (vaccine PRV strain) pigs going to slaughter if USDA permits.
 - III. Correlate tonsil biopsies from infected and vaccinated pigs with antibody response and titer.
- (b) Collect additional data on disease incidence, breeding, etc. for later evaluation.
- (c) Possible follow-up studies will be done in cooperation with TAHC, TVMDL, TAEX, Veterinary Microbiology, and Baylor College of Medicine as funds and personnel time are available from each organization.

6. General Herd Procedures

- (a) Disinfectant footbaths should be maintained in the main entrance to each separate area on the farm (breeding and gestation lots, farrowing house, farrowing house-nursery building, finishing area, incoming swine isolation area, reactor isolation area, and feed mill). The TAHC will bear the cost of furnishing an approved disinfectant.

- (b) Farrowing house crates and pens, nursery pens, finisher pens, and equipment and feeders will be thoroughly cleaned and disinfected between groups of swine. Finisher and breeding and gestation dirt lots are difficult to clean, but will be disinfected by sunlight and drying while unoccupied or as otherwise deemed appropriate. Feeders and other equipment in dirt lots will be cleaned and disinfected between groups of swine.
- (c) Every attempt will be made to eliminate contact between breeding animals (boars, sows, and gilts) and finishing animals. Herd selected replacement gilts will meet previously discussed testing requirements.
- (d) Every attempt should be made to prevent spread of pseudorabies to other livestock and pets (double fencing, etc.).
- (e) Any outbreak of clinical disease should be treated as possible pseudorabies and affected animals should be immediately isolated from the main herd while a diagnosis is attempted.
- (f) Dead animals that are not submitted for diagnostic workup should be promptly disposed of by burning or burial.
- (g) The PRV reactor isolation lot should be off limits to all but a designated employee and/or owner and should be located down wind (from prevailing wind direction) from the swine herd. Attention to footwear and equipment sanitation after leaving this isolation area is a must. This isolation lot should be located well away from any other swine in the main herd. Shipment to slaughter should proceed as soon as possible after identification of reactors.
- (h) All trailers used to move breeding stock, transport slaughter hogs or reactor breeding swine, or nurse hogs should be cleaned and disinfected between use in an area away from usual farm traffic.
- (i) Visitor traffic should be kept to a minimum and strict footwear sanitation should be followed at all times.
- (j) A stringent rodent control program should be followed.
- (k) Feral swine should continue to be eliminated as possible from the farm. Blood samples should be taken and tested for PRV as each feral pig is killed or trapped. The TAMC will continue to bear the expense for this testing.
- (l) Cats, dogs, and other pets should not be allowed to roam free through the herd. Stray dogs and cats should be eliminated.
- (m) Nocturnal wild animals (such as raccoons or skunks) that are found dead or seen wandering around in the daytime may have PRV and should be eliminated and/or properly disposed of.

STATE OF TEXAS §
 §
 COUNTY OF HARRIS §

KNOW ALL MEN BY THESE PRESENTS:

WAIVER AND RELEASE OF LIABILITY

I, the undersigned, do hereby request that NovaGene, Ltd., a Texas Limited Partnership (hereinafter referred to as "NovaGene"), vaccinate my herd of pigs with its modified live-virus pseudorabies vaccine. I fully understand that the NovaGene pseudorabies vaccine has recently been invented and developed, and the vaccine is still experimental. I voluntarily and with full informed consent assume all of the risk of any adverse consequence of the said vaccination. For good and valuable consideration, the receipt and sufficiency of which is hereby acknowledged, I hereby waive and release NovaGene, Ltd., Baylor College of Medicine, and all of their agents, consultants, employees, and partners, from any and all liability whatsoever, claim of liability, or any other allegation of damages, that may arise or pertain to the above-mentioned vaccination of the undersigned's herd of pigs with the NovaGene pseudorabies vaccine.

This waiver and release is binding upon the undersigned, and each and every person or party that may claim by or through the undersigned, including, but not limited to, successors in interest, subsidiaries, or other affiliates.

Agreed to and signed this ____ day of _____, 1984.

Texas Animal Health Commission

an equal opportunity employer

210 Barton Springs Road
P. O. Box 12966
Austin, Texas 78711-2966John W. Holcombe, DVM
Executive Director
512/475-4111

COMMISSIONERS

Robert F. Bartlett,
Chairman
Bob Barr
Laurence Shultz
R. M. Bolars, III
James H. Eller
C. E. Keady
Charles Koser
James B. Owen
Florence Dick
James B. Sarver
James Treder
Mary Ann West

June 5, 1984

Saul Kit, Ph.D.
Professor and Head
Division of Biochemical
Virology
Baylor College of Medicine
Texas Medical Center
Houston, Texas 77030

Dear Dr. Kit:

Upon review of the protocol for your experiment, we are granting permission to initiate the project with the following stipulations:

1. The Commission will fund serological tests at 30, 60, and 90 days as is its policy with all pseudorabies infected herds. Any subsequent testing will have to be granted after assessing the progress which has been made in controlling and eliminating pseudorabies in this herd.

Please complete the individual herd plan, obtain the necessary signatures and send a signed copy of this plan back to our office.

Sincerely,

John W. Holcombe, DVM
Executive Director

JWH:es



Celebrating Our Hundred and Fifty Years - 1934 - 1984

M. H. H. L.
unannounced by
Dr. Latham
June 26, 1984

May 31, 1984

John W. Holcombe, D.V.M.
Executive Director
TEXAS ANIMAL HEALTH COMMISSION
San Houston State Office Building
P. O. Box 12966
Austin, TX 78711-2966

Dear Dr. Holcombe,

Enclosed is a proposed pseudorabies vaccination experiment for the Wallace and Tommy Maddox swine herd near Loneta. This plan incorporates interim vaccination with a new experimental pseudorabies virus vaccine developed by Drs. Saul Kit and Malon Kit in the laboratories of the Division of Biochemical Virology, Baylor College of Medicine, Houston, Texas, under the sponsorship of NovaGene, Ltd., Houston, Texas. The experimental herd plan for the use of this new vaccine has been devised with the advice of Dr. Bruce Lawhorn. The herd owners have agreed to its implementation.

If the plan meets with your approval, we request permission to proceed with the experiment.

Thank you for your time.

Sincerely yours,

Saul Kit, Ph.D.
Professor and Head,
Division of Biochemical Virology

SK:gb

Enclosures

cc Dr. Bruce Lawhorn

PSEUDORABIES VACCINATION EXPERIMENT FOR WALLACE AND TORDY MADDOX HERD

The purpose of this vaccination experiment is to eliminate pseudorabies from this herd in the most practical and economical way possible with the least loss of livestock. A live experimental pseudorabies vaccine will be used to attempt blockage of natural infection spread from infected to noninfected animals within the herd or from possibly infected feral swine to noninfected herd swine. The use of this vaccine is necessary because the current test and removal herd plan has not successfully contained virus spreading. In order to attempt pseudorabies elimination from this herd, the following steps will be taken:

1. Experimental Vaccination

- (a) All pigs on the premise on Day 0 regardless of age, sex, or other criteria will be vaccinated IM with a single 2 ml pseudorabies (PRV) virus (BHK-d1 3B) containing 10^7 pfu (pfu = one virus particle).
- (b) Sows that farrow within three to four weeks post vaccination (through Day 30) will be devoid or have reduced PRV maternal antibody to protect piglets from natural infection. Vaccine virus is not thought to spread from vaccinated females to piglets protected or unprotected by maternal PRV antibody. Therefore, all possibly unprotected piglets farrowed through Day 30 should be vaccinated as soon as possible after birth.
- (c) Any piglets through Day 90 that do not receive adequate maternal PRV antibody, as indicated by becoming poor-doe pigs may be totally unprotected from WT PRV infection and possibly spread the virus if infected. These poor-doe pigs should be immediately vaccinated or killed and disposed of by burning or deep burial.
- (d) Anticipated requirements for this study are 1500 doses of vaccine (10^7 pfu) to be given as a single 2 ml dose IM. The Maddox herd contains approximately 175 breeding animals (sows, gilts, and boars) and 1200 growing and finishing pigs at any one point in time. The farrowing and finishing areas are separated, but some breeding and gestation lots are in close proximity to finishing lots. Animal flow is one-way from the farrowing area to finishing except for replacement herd gilts which are returned to breeding and gestation lots. All boars are obtained from off the premises.
- (e) All vaccinated animals are to be marked with a hole punched in the left ear of not less than $\frac{1}{4}$ " in diameter at the time of vaccination. This hole punch instrument will be provided by the Texas Animal Health Commission. All swine in this vaccinated herd will be quarantined to this premise and may be moved only to slaughter.

(f) Product description-pseudorabies virus with the virulent genetic code seared by enzymatic excision. This virus has been developed in the laboratory of Dr. Saul Kit, Department of Biochemical Virology, Baylor College of Medicine, Houston, Texas. Preliminary testing in pigs and calves has shown this virus isolate to be safe and immunogenic. At the present, only limited quantities of this virus are available and no commercial production is anticipated in the immediate future.

(g) Product liability disclaimer-see Attachment 1.

2. Initial Serology

- (a) On Day 0 (day of PRV vaccination) all breeding swine (boars, sows, and gilts including newly selected finishing lot replacements) will be blood sampled for PRV antibody. Individually cleaned and disinfected syringes and needles will be used to sample each animal for testing. The portion of each restraint snare contacting an animal during bleeding will be disinfected before snaring another pig. Footwear will be disinfected before entering each separate lot of swine.
- (b) Personnel of the Texas Animal Health Commission (TAHC) will draw blood for testing. All testing will be done at the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) in College Station or Amarillo and all tests will be done by the serum virus neutralization test (SN test). Testing expense will be borne by the TAHC.
- (c) All Day 0 PRV antibody positive swine will be moved to an isolated reactor lot and shipped to market as soon as possible. Such positive swine are considered latently infected with wild type (WT) PRV and may spread this virus especially if stressed.
- (d) All Day 0 PRV antibody negative swine will be identified as noninfected vaccinates. They will be considered as nonlatent noninfected WT PRV swine pending further testing.

3. Post-Vaccination Serology

- (a) On Days 30, 60, and 90 and any additional approximate 30 day intervals thereafter, post-vaccination breeding herd replacement blood samples will be taken.
- (b) All tested pigs with PRV SN titers of greater than or equal to 1:16 will be considered as suspicious. These animals will be moved to an isolated reactor lot and shipped to market as soon as possible.
- (c) All tested pigs with PRV SN titers of less than or equal to 1:8 will be considered as non-infected vaccinates and be retained within the herd.

- (d) All vaccinated gilts that are selected for breeding stock must have PRV SN titers of 1:8 or less and must not have exhibited PRV signs. This procedure may require additional testing other than on Days 30, 60, 90, etc.
- (e) Prior to introduction into the main herd, all new boars (or any new breeding stock) must be isolated from other swine on the premise for at least 30 days (60 days is better). They also must be tested negative for PRV at least once toward the end of this isolation period.

4. Sentinal Animals

Pigs born to vaccinated sows after Day 30 should be protected by vaccine-induced maternal PRV antibody (provided they nurse properly). These pigs will not be vaccinated and will serve as sentinal pigs after maternal immunity wears off by twelve to eighteen weeks of age.

5. Possible Follow-Up Studies

- (a) Virus study-collect tonsils and trigeminal nerve (right gasserian ganglia) for viral DNA probes to determine latency and type of virus colonizing the tissues.
 - i. Collect tissues for virus isolation from pigs that are aborted and/or die on the premise.
 - ii. Collect tissues from selected WT PRV and PRV-dl (vaccine PRV strain) pigs going to slaughter if USDA permits.
 - iii. Correlate tonsil biopsies from infected and vaccinated pigs with antibody response and titer.
- (b) Collect additional data on disease incidence, breeding, etc. for later evaluation.
- (c) Possible follow-up studies will be done in cooperation with TAMC, TWHC, TALE, Veterinary Microbiology, and Baylor College of Medicine as funds and personnel time are available from each organization.

6. General Herd Procedures

- (a) Disinfectant footbaths should be maintained in the main entrance to each separate area on the farm (breeding and gestation lots, farrowing houses, farrowing house-nursery building, finishing area, incoming swine isolation area, reactor isolation area, and feed mill). The TAMC will bear the cost of furnishing an approved disinfectant.



- (b) Farrowing house crates and pens, nursery pens, finisher pens, and equipment and feeders will be thoroughly cleaned and disinfected between groups of swine. Finisher and breeding and gestation dirt lots are difficult to clean, but will be disinfected by sunlight and drying while unoccupied or as otherwise deemed appropriate. Feeders and other equipment in dirt lots will be cleaned and disinfected between groups of swine.
- (c) Every attempt will be made to eliminate contact between breeding animals (boars, sows, and gilts) and finishing animals. Herd selected replacement gilts will meet previously discussed testing requirements.
- (d) Every attempt should be made to prevent spread of pseudorabies to other livestock and pets (double fencing, etc.).
- (e) Any outbreak of clinical disease should be treated as possible pseudorabies and affected animals should be immediately isolated from the main herd while a diagnosis is attempted.
- (f) Dead animals that are not submitted for diagnostic workup should be promptly disposed of by burning or burial.
- (g) The PRV reactor isolation lot should be off limits to all but a designated employee and/or owner and should be located down wind (from prevailing wind direction) from the swine herd. Attention to footwear and equipment sanitation after leaving this isolation area is a must. This isolation lot should be located well away from any other swine in the main herd. Shipment to slaughter should proceed as soon as possible after identification of reactors.
- (h) All trailers used to move breeding stock, transport slaughter hogs or reactor breeding swine, or nursery pigs should be cleaned and disinfected between use in an area away from usual farm traffic.
- (i) Visitor traffic should be kept to a minimum and strict footwear sanitation should be followed at all times.
- (j) A stringent rodent control program should be followed.
- (k) Feral swine should continue to be eliminated as possible from the farm. Blood samples should be taken and tested for PRV as each feral pig is killed or trapped. The TAMC will continue to bear the expense for this testing.
- (l) Cats, dogs, and other pets should not be allowed to roam free through the herd. Stray dogs and cats should be eliminated.
- (m) Nocturnal wild animals (such as raccoons or skunks) that are found dead or seen wandering around in the daytime may have PRV and should be eliminated and/or properly disposed of.

- (m) Open feed mill augers should be covered with screening to prevent "bedding down" areas for possibly infected cats, rodents, or wild animals. Mixers should be inspected periodically for "hung up" animal carcasses. Commercial feed delivery vehicles should have the wheels and tires disinfected before entering the premise. Feed delivery personnel should not be allowed to roam free in the feed mill and must follow strict footwear sanitation.
- (o) Equipment used on reactor animals will either be cleaned and disinfected before using or not used on "clean" animals.
- (p) This experimental plan will be re-evaluated and changed when a change in circumstances is evident to the State Epidemiologist. This will include when the herd is not progressing toward cleanup, and it will be the responsibility of all parties to the agreement to adhere to the plan throughout the period of eradication of this disease from this herd.

7. Quarantine Requirements and Term

- (a) All vaccinated, non-vaccinated, and infected animals in this herd are under quarantine and, until such time as the quarantine is removed, will be shipped only directly to slaughter under permit.
- (b) As long as vaccinated swine (test positive or negative) remain in this herd, premise quarantine will remain in force.

8. Quarantine Release Requirements

- (a) All vaccinated and test positive swine must be removed from the premise; and,
- (b) There have been no clinical signs of pseudorabies on the premises after removal of the vaccinated and test positive swine; and
- (c) All exposed swine which remain in the herd have withstood two consecutive negative herd tests, the first being not less than 30 days from removal of the last test positive or vaccinated animals and the second test coming not less than 30 days from the first test.

This plan is agreed upon the 26 day of June, 1980.

Paul Kit
Baylor College of Medicine
Paul Kit, Ph. D.
Professor and Head
Division of Biochemical Virology

Bruce Lawhorn, D.V.M.
The Texas A&M University System
Bruce Lawhorn, D.V.M.
Extension Swine Veterinarian

Wallace Maddox, Owner

Tommy Maddox, Owner

James Maddy

STATE OF TEXAS §
COUNTY OF HARRIS §

KNOW ALL MEN BY THESE PRESENTS:

WAIVER AND RELEASE OF LIABILITY

I, the undersigned, do hereby request that NovaGene, Ltd., a Texas Limited Partnership (hereinafter referred to as "NovaGene"), vaccinate my herd of pigs with its modified live-virus pseudorabies vaccine. I fully understand that the NovaGene pseudorabies vaccine has recently been invented and developed, and the vaccine is still experimental. I voluntarily and with full informed consent assume all of the risk of any adverse consequence of the said vaccination. For good and valuable consideration, the receipt and sufficiency of which is hereby acknowledged, I hereby waive and release NovaGene, Ltd., Baylor College of Medicine, and all of their agents, consultants, employees, and partners, from any and all liability whatsoever, claim of liability, or any other allegation of damages, that may arise or pertain to the above-mentioned vaccination of the undersigned's herd of pigs with the NovaGene pseudorabies vaccine.

This waiver and release is binding upon the undersigned, and each and every person or party that may claim by or through the undersigned, including, but not limited to, successors in interest, subsidiaries, or other affiliates.

Agreed to and signed this 26 day of June, 1984.

Thomas P. Phillips

John W. Phillips

John W. Phillips

Exhibit D-3

Letter from Bruce Lawhorn, DVM, Extension Swine Veterinarian,
Texas Agricultural Extension Service to Dr. John Holcombe
dated August 16, 1984

6 AUG 1984
TAMU

**TEXAS
Agricultural
Extension
Service**

The Texas A&M University System
College Station, TX 77843
818 Veterinary Medical Administration Building
August 16, 1984

Dr. John Holcombe
Texas Animal Health Commission
Post Office Box 12966
Austin, Texas 78711

Dear Dr. Holcombe:

Enclosed are the results of the 30 day test following whole herd experimental vaccination of the Maddox pseudorabies quarantined herd. Two hundred eleven breeding animals (including 22 replacement gilts of unknown PRV serostatus) were sampled in two days. Novogene, LTD paid for these 211 SN tests. Many interesting and exciting possibilities are emerging from this experiment and are as follows:

1. The dramatic and consistent anamnestic antibody rise following experimental vaccination of the pen of 22 replacement gilts (see page 4 and 5 of 7/27/84 result sheet enclosed) strongly indicates these gilts were incubating or infected with wild-type pseudorabies virus prior to vaccination. Also these gilts are housed between other pens of finishing pigs that were farrowed at the time the original Maddox PRV outbreak occurred. This means that even though the main herd of 185 breeding swine tested negative for PRV (by SN test) on approximately 6/8/84 (about 70 days after the previous breeding herd test with 14 positives), pseudorabies virus was still circulating in the finishing pens (as Dr. Fancher and I expected). Also during a recent visit to this farm, Wallace Maddox told me he counted 70 wild-type crossbred pigs (identifiable by conformation and growth stallout at 120 to 140 pounds) dispersed throughout all finishing pens. It is clearer now that in this herd these remaining possible sources for PRV infection would make it risky to expect a test, isolation, and slaughter herd plan (without vaccination) to have provided long-lasting relief from pseudorabies reinfection and re quarantine.
2. All non-infected breeding swine (tested negative on 6/8/84) had no higher than a 1:4 SN PRV antibody titer 30 days post vaccination. This means that the cut-off titer point of $\leq 1:8$ for negative vaccines was correctly predicted by the experimental design.

Dr. H. L. Wallace
Page 2

Seventy-two of 189 vaccinates (not counting 22 high titered gilts) had either a 1:2 or 1:4 antibody titer. The remaining 117 that showed no SN antibody titer rise at this 30 day test may exhibit a titer on the 60 day test (Novagene has agreed to pay for the second test). The lack of a titer does not necessarily mean these animals are not protected from field challenge. Laboratory research has shown that vaccinated animals are well protected, but actual challenge studies in Maddox animals could be the final answer to this.

The pattern of distribution of titered and negative vaccinates throughout our testing indicates that the vaccine was correctly handled and administered. Follow-up evaluation of vaccine not used and refrozen following two days of vaccination is expected to verify this assumption.

3. In the future, the vaccine used in this experiment may furnish a one-injection barrier to the spread of PRV during an outbreak situation. This means such a vaccine may easily fit any management situation and still furnish the flexibility of distinguishing infected vaccinates from non-infected vaccinates.
4. The use of this vaccine in the Maddox herd and other experiments has shown its safety. No identifiable adverse reactions have been noted by Wallace, Tommy, Jimmy, or me. Pregnant sows (in all stages of pregnancy), boars, and pigs as young as one day old have been vaccinated. Approximately 1400 doses of vaccine were used in the Maddox herd.

In summary, I feel very positive about the use of this experimental PRV vaccine in the Maddox herd and express my appreciation to you for approving its use in this ongoing experiment.

Sincerely,

Bruce Lawhorn

Bruce Lawhorn, DVM
Extension Swine Veterinarian

BL:cb

Enclosure

cc: Dr. Zerie Carpenter
Dr. Walter Walla
Dr. George Shelton
Dr. Saul Kit ✓
Dr. F. C. Farles
Dr. Charles Boyd
Dr. Thurman Fancher

Exhibit D-4

**"Pseudorabies Vaccination-Eradication Experiment
Utilizing a Modified-Live, Deletion Mutant Virus Vaccine"
by Bruce Lawhorn, DVM**

*Presented at the Meeting
of March 15, 1984*

**Pseudorabies Vaccination-Eradication Experiment Utilizing A
Modified-Live, Deletion Mutant Virus Vaccine**

Bruce Lawhorn, DVM*
Extension Veterinarian
Texas Agricultural Extension Service

One usual method for pseudorabies (PRV) eradication in PRV quarantined herds has traditionally been the test, removal, and slaughter technique combined with other management techniques to limit PRV spread. The failure to prevent PRV spreading between testing intervals has frequently led to the gradual depopulation of quarantined herds. Purebred and commercial producers may suffer extreme economic losses from such gradual depopulation due to loss of valuable bloodlines and disrupted cash flow caused by dwindling breeding herd inventories. In areas of Texas where PRV-infected wild or feral swine populations exist, these possible sources for PRV exposure may persist during and after quarantined herd clean-up efforts.¹ An additional barrier to PRV spread within a quarantined herd is vaccination. Any PRV vaccine used should be safe, effective, and not interfere with interpretation of blood test results. Just as important, any PRV vaccination program must easily fit into the management scheme of most producers. An experimental modified-live, deletion mutant PRV vaccine was used in one western Central Texas PRV quarantined herd to determine if all of the aforementioned criteria could be met.

Outbreak History

On February 16, 1984, owners of a 185 sow farrow-to-finish operation

* Taken in part from a Thesis to be submitted to the Graduate College, Texas A&M University, in partial fulfillment for the degree of Masters of Science.

brought 4, two-week-old pigs to the Texas Veterinary Diagnostic Laboratory (TVMDL) in College Station. The two pigs arriving alive were gaunted up, shaking, and had temperatures exceeding 108°F (42.2°C). Nearly all pigs in six litters had been affected during the previous week. Clinical signs seen in these pigs were shaking followed by death within 2 or 3 days. Diagnosis of PRV was conclusively made by histopathology, virus isolation, fluorescent antibody identification, and PRV cytopathologic effect in cell culture. The herd was then quarantined and all off-premise movement restricted except directly to slaughter.

Two possible sources for PRV exposure were breeding animals introduced several months previous and periodic feral pig contact with the breeding herd. The owners commented that 2 of the pigs submitted for laboratory analysis had coloration indicating feral boars were their sires. Many crossbred feral pigs, identifiable by growth stallout, were also scattered throughout the grower-finisher pens.

Initial Herd Testing

One hundred seventy-three breeding animals were first blood sampled on February 24, 1984, by the Texas Animal Health Commission (TAHC). Twelve sows were seropositive and removed to an isolation pen prior to slaughter. Litters from positive sows were also separated from adjacent negative sow litters prior to and after weaning. None of the recently introduced breeding swine tested seropositive.

Prior to the next breeding herd test, shaker pigs continued to sporadically occur, and 4 more litters were affected. Thirty days following the first breeding herd test, 13 sows were seropositive on the second herd test. These

sows were also sent to slaughter. Periodic testing of replacement gilts from the finishing area had been producing negative results, but prior to the third breeding herd test, one seropositive replacement gilt indicated PRV could be spreading through the finishing area.

Although the third breeding herd was delayed about 70 days from the second, all 185 animals tested were seronegative. At this point the breeding herd appeared to be free of PRV. However, the threat of continual exposure from possible PRV carrier feral swine and the likely circulation of PRV in approximately 1,000 nursery and grower-finisher pigs near the "clean" breeding herd made an experimental vaccination barrier worthy of consideration.

Experimental Vaccine

A thymidine kinase (TK)-negative (TK-) deletion mutant of the Bucharest (BUK) strain of PRV was isolated in the Baylor College of Medicine Molecular Virology Laboratory of Dr. Saul Kit.² The mutant virus was designated as PRV (BUK d13).² Dr. Kit, cooperating with Dr. Stewart McConnell of Texas A&M University, made the appropriate doses of experimental vaccine (10^7 PFU/ml) from this TK- PRV deletion mutant virus (BUK d13).

Ideally, protection from natural PRV infection would theoretically occur when non-infected, vaccinated pigs blocked any further PRV infection following field exposure. The potential for impeding virulent virus spread in infected swine herds has been suggested by intramuscular TK- PRV (BUK d13) vaccination and virulent virus challenge studies.² Immunization of swine with TK- PRV (BUK d13) has been shown to protect pigs from clinical signs of disease when challenged with large doses of virulent PRV.² The amount and duration of oropharyngeal shedding after virulent virus challenge is also reduced.² Latency does not appear to be established following vaccination of swine with TK- PR

(BUK d13).² Also, immunization with TK- PRV (BUK d13) virus does not totally prevent persistent tonsillar infection by virulent PRV.²

The safety of TK- PRV (BUK d13) virus has been experimentally demonstrated in mice, rabbits, sheep, calves, and pigs, and will not revert to TK positive in vitro.^{2,3} The TK- phenotype and other biochemical properties of this virus make it distinguishable from other PRV strains.²

Texas Animal Health Commission Approval of Experimental Vaccination

With the cooperation of Wallace Maddox and Sons (herd owners), the Texas Agricultural Extension Service (TAEX), the TAHC, the Texas Pork Producers Association, the TVMDL, Dr. Stewart McConnell, and Dr. Saul Kit, a written plan for the PRV vaccination/eradication experiment was submitted for approval. Following acceptance by Dr. John W. Holcombe, Executive Director of the TAHC, the plan went into effect.

Safety Study

The purpose of this study was to evaluate the safety of TK- PRV(BUK d13) vaccine in newborn pigs. A 3-day-old female, a 3-day-old castrated male, and a 5-day-old castrated male pig were used. The 3-day-old pigs were littermates. All pigs were Yorkshire x Duroc x Landrace x Hampshire crosses. All pigs had received colostrum milk from sows obtained from a Qualified, PRV-free herd and 100 mg of intramuscular iron dextran before this study.

Pigs were weaned and housed in cardboard boxes. The 3-day-old pigs were kept in one box, and the 5-day-old pig was kept in another. The boxes were located in a closed environment where the daytime high temperature was approximately 100°F (38°C) and the nighttime low was approximately 75°F (24°C). Pigs were housed in boxes to prevent draft exposure and aid conversion to a

substitute diet. The diet consisted mainly of 2 or 3 percent pasteurized cow's milk fed four to five times per day.

The 5-day-old castrated male pig and the 3-day-old female pig were inoculated in the neck muscle with 2 ml (10^7 PFU/ml) of TK- PRV (BUK d13) vaccine. The castrated male 3-day-old littermate served as a contact control. The rectal temperature of the pigs was followed after inoculation only, since the high environmental temperature and the stress of shipment on the inoculation day caused all pigs to salivate profusely and to breathe rapidly until they cooled down that night. Although the rectal temperature fluctuated, the 5-day-old castrated male pig at day one post-inoculation was the only pig of the immunized group to exhibit any elevation of temperature (103.2°F ; 39.6°C).

The pigs took about 24 hours to convert to the substitute diet. Once they started drinking milk, they never lost their appetites. The 3-day-old pigs experienced a mild diarrhea, possibly from consuming excessive milk. No antibiotics were used in treatment, so if infection was present, it was mild. The three pigs reached market weight and were sent to slaughter.

Previous studies have also demonstrated the safety of TK- PRV (BUK d13) vaccine.^{2,3}

Herd Vaccination

On June 26 and 27, 1984, approximately 1275 pigs of all ages were inoculated intramuscularly with 2ml (2×10^7 PFU) of TK- PRV (BUK d13) vaccine and ear notched. Approximately 125 pigs from sows within 3 weeks or less of farrowing at initial vaccination were thought unprotected by anti-PRV colostral antibody and were vaccinated and ear notched on July 18. Unvaccinated pigs farrowed after July 18 were to serve as sentinel pigs to indicate any subsequent

natural PRV spread.

Testing the breeding herd and replacement gilts and boars at 30-day intervals post-vaccination was designed to separate naturally infected and non-infected vaccinates and monitor natural PRV spread. As long as any vaccinated and/or infected pigs remained on the premise, the herd would remain quarantined.

Thirty-Day Post-Vaccination Results

According to previous research data, non-PRV-infected pigs inoculated once with TK- PRV (BUK d13) vaccine had serum virus neutralization (SN) titers of 1:16 or less.³ Pigs naturally infected or incubating PRV were expected to have a significant "boosted" titer. Based on these expectations, a titer margin of error was added and the experimental design was set up to classify titers of 1:8 or less as non-infected vaccinates and titers of 1:16 or more as naturally exposed or infected vaccinates.

On July 26 and 27, 1984, the TAEX and the TAHC cooperated to blood sample 211 breeding animals 29 to 31 days after whole herd vaccination. One hundred eighty-nine breeding animals previously SN-tested negative on the third test prior to vaccination now had SN titers of 1:4, 1:2, or negative (at 1:2). Twenty-two vaccinated replacement gilts never before tested and recently selected from the "suspect" finishing pens had 1:16 to 1:128 titers (see Table I). These results indicated that the experimental design had correctly predicted the titer dividing point between previously non-infected vaccinates and infected or exposed vaccinates.

Sixty-Day Post-Vaccination Test Results

On August 27 and 28, 1984, 213 animals were blood-sampled 61 to 63 days following whole herd vaccination. Fifty-eight of 184 previously vaccinated

breeding animals had SN titers of 1:2 or 1:4, including 19 which had been SN negative on the thirty-day post-vaccination testing (see Table I). Five of 22 replacement gilts never before tested and selected from the "suspect" finishing pens had titers of 1:16 to 1:32. All but four of the other 17 had titers of 1:2 to 1:8. The 5 gilts with anamnestic responses were isolated to a quarantine pen prior to shipment and slaughter. The gilt with the 1:8 titer was also removed as insurance. In addition, the relatively lower number of 60-day test gilts with 1:16 or greater titers compared to the 22, 1:16 or greater titers on 30-day test gilts could be field evidence for vaccine effectiveness.

At the time of the 60-day test, 1 vaccinated 8-week-old pig had a titer of 1:4. This pig's dam had been vaccinated less than 1 week before farrowing. There were also five 2 to 4-week-old uninoculated pigs from vaccinated sows that had had 4 to 6 weeks to develop antibodies prior to farrowing. None of the 5 pigs had detectable SN titers (negative at 1:2), suggesting that pigs less than 2 weeks old, even originating from vaccinated sows, can be immunized.

On October 14, 1984 (110 days post-vaccination), 5 of 14 vaccinated, replacement gilts had titers of 1:16, and the rest had titers of 1:2 to 1:8. The 1:16 titered gilts were isolated prior to shipment and slaughter. All 14 samples were also positive by enzyme-linked immunosorbent assay (ELISA).

On October 26, 1984, one feral boar was killed near the breeding herd on the Maddox farm. Serum submitted from this boar was SN positive at 1:4 and ELISA positive.

On January 17, 1985, sera submitted from a feral boar and sow killed on the Maddox farm tested ELISA positive.

Two Hundred Forty-Eight-Day Post-Vaccination Results

On March 11, 1985, 240 breeding animals were tested. Of these, 53

vaccinated animals had titers of 1:2 to 1:4, one had 1:8, and one had a 1:16 titer (see Table I). The 1:16 titer gilt was identified as a previously infected, vaccinated animal that was not sent to slaughter. No unvaccinated animal had a positive SN titer.

On August 1, 1985, 14 unvaccinated replacement gilts tested SN negative.

Field Vaccine Safety

No identifiable adverse reactions have occurred in this herd after approximately 1,400 doses of vaccine were administered. Females in many stages of pregnancy (see Table II), boars, and pigs as young as 1 day of age (see Table III) were vaccinated. These field safety observations support laboratory evidence for vaccine safety in pigs.

Summary

The experimental TK- PRV (BUK d13) vaccine used appears to be extremely safe and did not interfere with SN test interpretation from 30 to 248 days post-vaccination. Identification of naturally exposed or infected animals by anamnestic responses was also accomplished following immunization. The vaccination schedule of one injection given to all pigs regardless of age easily fit into this producer's management scheme. The vaccine also created a potential barrier against natural PRV spread from within the herd and/or from possibly PRV-infected feral pigs outside the herd. It also appears that the duration of immunity from one vaccination may be at least 248 days.

References

1. Lawhorn, D.B., 1984. Pseudorabies and Brucellosis in East Texas Piney Wood

- Rooter Hogs. Texas Pork Producer. July/August, 4.
2. Kit, S., Kit, M., Pirtle, E.C., 1985. Attenuated properties of thymidine kinase-negative deletion mutant of pseudorabies virus. Am. J. Vet. Res. 46:1359-1367.
 3. Kit, S., Kit, M., Lawhorn, D.B., McConnell, S., 1985. Immunization of Pregnant Pigs in a Quarantined Swine Herd with a Thymidine Kinase Deletion Mutant of Pseudorabies Virus. High-Technology Route to Virus Vaccines. American Society for Microbiology. Washington, D.C., 82-99.

Table 2. Antibody formation by breeding swine after 12-PRV (BHK 213) vaccination

Specimen ^a	Titer				Specimen	Titer				Specimen	Titer				Specimen	Titer			
	30 day	60 day	240 day			30 day	60 day	240 day			30 day	60 day	240 day			30 day	60 day	240 day	
16	2	2	2		21	4	4	N		224	2	2	NS		41	2	2	N	
17	2	NS ^b	4		209	2	2	NS		214	2	2	N		50	2	N	NS	
Tube #4	4	4	NS		225	2	4	NS		231	N	2	NS		179	N	2	N	
18	2	N	N		117	2	N	N		240	4	NS	N		176	2	2	NS	
20	N	4	NS		91	2	4	NS		236	4	2	N		17	2	2	N	
26	N	4	N		80	4	2	2		238	4	N	N		12	4	N	N	
48	2	N	N		245	64	NS ^d	NS		243	4	2	NS		177	N	2	2	
11	N	2	NS		246	32	0	NS		40	4	4	4		18	2	2	N	
40	4	2	N		247	64	0	NS		242	2	N	N		13	N	2	N	
72	2	N	2		248	128	0	NS		234	2	NS	N		7	4	2	4	
79	4	4	4		249	8	0	NS		180	2	2	4		8	2	4	NS	
33	4	4	N		252	64	0	NS		175	N	4	NS		92	N	2	NS	
216	2	N	4		253	128	0	NS		224	4	NS	N		51	N	2	N	
217	4	4	8		254	64	0	NS		228	4	2	N		121	2	N	N	
294	4	2	N		255	32	0	NS		230	4	NS	4		164	4	NS	NS	
297	N	2	N		256	64	0	NS		233	4	4	4		173	N	2	N	
298	4	4	4		257	32	0	NS		239	2	2	NS		102	2	2	2	
73	4	4	NS		258	32	0	NS		197	N	2	N		1A	NS ^e	320	NS	
118	2	N	NS		259	64	0	NS		196	4	NS	NS		2A	NS	4	NS	
210	4	2	NS		260	16	0	NS		187	N	2	N		3A	NS	4	NS	
129	2	N	NS		261	64	0	NS		178A	N	2	NS		4A	NS	N	NS	
223	4	NS ^c	N		262	64	0	NS		22	2	N	N		5A	NS	160	NS	
113	N	2	N		263	64	0	NS		192	2	NS	NS		6A	NS	160	NS	
131	4	NS	NS		264	128	0	NS		190	N	2	2		7A	NS	80	NS	
122	2	N	N		174A	64	0	NS		195	N	2	N		8A	NS	4	NS	
106	2	N	N		175A	128	0	NS		163	2	N	N		9A	NS	2	NS	
124	2	N	NS		176A	32	0	NS		57	N	2	NS		10A	NS	4	NS	
212	4	4	NS		177A	32	0	NS		29	4	2	NS		11A	NS	2	NS	
128	4	2	NS		227	4	2	NS		115	4	2	N		12A	NS	N	NS	
94	2	N	NS		219	4	2	N		3	2	N	2		13A	NS	320	NS	
229	2	N	NS		215	4	4	NS		83	4	4	4		14D	NS	N	NS	
					237	4	4	NS		54	2	N	NS		15A	NS	320	NS	
										32	2	N	N		16A	NS	2	NS	
															17A	NS	4	NS	
															18A	NS	4	NS	
															19A	NS	N	NS	
															20A	NS	4	NS	
															21A	NS	4	NS	
															22A	NS	N	NS	

^a Specimen No. is ear tag No. unless specified otherwise^b N signifies negative^c NS signifies no sample^d 0 signifies gilt moved to isolation pen and sent to slaughter^e ND signifies not done

Table II. Characteristics of litters from sows immunized with TK-99V (BHK d13)

Description of immunized sows	Total no. of litters	Total no. of pigs born alive	Avg. litter size born alive	Total no. of pigs weaned ^a	Avg. weaned	% (weaned/born alive)	Avg. stillbirths per litter	Avg. mummified per litter	Date of farrowing and location
Control sows: immunized 1 to 2 weeks after farrowing; 95 pigs immunized on same day ^b	12	129	10.75	92	7.66	71.3	NA ^c	0.33	June & July, NFH
Immunized 4 to 4 weeks pre-farrowing; two litters immunized at 3 weeks and six not vaccinated	8	91	11.38	73	9.13	80.2	1.25	0	July, NFH
Immunized 34 to 49 days pre-farrowing ^d	17	190	11.18	160	9.41	84.2	0.47	0	July & Aug. NFH (12), TFH (5)
Immunized 55 to 64 days pre-farrowing ^d	15	143	9.53	135	9.00	94.4	2.26 ^f	0	Aug. thru 16 Sept., TFH
Immunized 68 to 77 days pre-farrowing ^d	19	NA ^g	NA	168	8.84	NA	NA	NA	Sept., NFH
Immunized 85 to 104 days pre-farrowing ^d	14	148	10.57	129	9.21	87.0	1.21	0	26 Sept. to 10 Oct. TFH

^a Some piglets lost by trauma or mothers were poor milkers^b The mortality of two vaccinated pigs was due to exudative epidermitis, and one pig died of small unthrifty pig syndrome.^c NA, Not available^d NFH, New farrowing house, Wallace Maddox farm; TFH Tommy Maddox farrowing house.^e Litters not immunized.^f Peaked with height of summer heat.^g NR, Not recorded.

Table III. Characteristics of newborn pigs immunized with TK-PRV (DUK d13)

Description of pigs	No. immunized	No. surviving and weaned	Location	Adverse Reactions
Immunized at ≤ 1 to 2 weeks of age; from immunized sows	208	204 ^a	TFH (113), ^b NFH (95)	None observed
Immunized at 3 weeks of age; from immunized sows	16	16	NFH	None observed

^a Of the four pigs that did not survive to weaning, two developed exudative epidermitis. One was a very small pig. The reason for the death of the fourth pig was unknown.

^b TFH, Tommy Maddox farrow house; NFH, new farrow house on Wallace Maddox farm.

PART FIVE

Relationships of Dr. Saul Kit with TECHAMERICA GROUP, INC. and
with the USDA as it Relates to the USDA Licensing of the Vaccine

Relationships of Dr. Saul Kit with TECHAMERICA GROUP, INC. and with the USDA as it Relates to the USDA Licensing of the Vaccine

I had no direct role in the process by which the USDA licensed TECHAMERICA GROUP, INC. to manufacture and sell OMNIVAC-PRV. After having supplied seed virus to TECHAMERICA GROUP, INC., I participated only indirectly in the USDA licensing process.

I transmitted all published and unpublished data available to me to TECHAMERICA GROUP, INC. so that the data would be transmitted to the USDA. For example, copies of U.S. Patent No. 4,514,497 were sent to TECHAMERICA GROUP, INC. for transmittal to the USDA.

Copies of USDA licensing correspondence and test results were forwarded to me by TECHAMERICA GROUP, INC. so that I could be appraised of progress and monitor the USDA licensing procedure.

Together with Dr. Stewart McConnell and Dr. Malon Kit, I was asked by TECHAMERICA GROUP, INC. to fly to Galesburg, Illinois, for a meeting with Thomas Miller and Dr. Michael Bartkoski (TECHAMERICA GROUP, INC.), Drs. Brad J. Thacker (Michigan State University), Roger Saline, Jorge Gonzalez (Swine MAP, Inc.), Drs. Spencer and Doby (State of Illinois Veterinarians), and Dr. Jelleck (State of Illinois Virus Diagnostic Laboratory). At this meeting, held on July 28-29, 1985, I explained in detail the properties of OMNIVAC-PRV and its construction.

During the USDA prelicensing period and after the USDA license was issued, TECHAMERICA GROUP, INC. routinely sent to us portions of each production lot for quality control. The quality control was carried out by verifying by restriction nuclease fingerprinting and/or thymidine plaque autoradiography that the viruses being sold by TECHAMERICA GROUP, INC. were authentic TK PRV deletion mutants.

PART SIX

Guidelines of the National Institutes of Health
Recombinant DNA Advisory Committee

Guidelines of the National Institutes of Health
Recombinant DNA Advisory Committee

All studies carried out in our laboratory at the BAYLOR COLLEGE OF MEDICINE were registered with the Biosafety Office of the Baylor Recombinant DNA Subcommittee and the Baylor Vivarium Biosafety Subcommittee (see: Exhibit F-1). The experiments were conducted in accordance with the NIH guidelines.

Experiments on the TK⁻ PRV(BUK-dl 3) (OMNIVAC-PRV) virus have been registered as Pl-EK1, Class II agent experimentation. The National Institute of Health Recombinant DNA Advisory Committee Guidelines, Paragraph III-D-2, 49 FR 46269 (1984) (see: Exhibit F-2) states that these experiments are exempt from the Guidelines:

III-D-2 - Those (DNA molecules) that consist entirely of DNA segments from a single nonchromosomal or viral DNA source though one or more of the segments may be a synthetic equivalent.

Our laboratory has also been guided by USDA Veterinary Services Memorandum No. 800.68, dated December 4, 1984 (see: Exhibit F-3), which states that:

In normal husbandry and laboratory practices, veterinary biological products are not considered to be released into the environment. (Emphasis added)

Our studies in Texas were conducted with the approval of the Texas Animal Health Commission and with the collaboration of Texas Extension Veterinarians. In an environment populated by feral pigs seropositive for pseudorabies and farm animals infected with pseudorabies, we believe that it is safe and appropriate to prevent disease by vaccinating pigs with a vaccine developed from another vaccine strain that had already been used in the U.S.A. since 1977. Again, the new vaccine OMNIVAC-PRV is safer than the original one.

We and others do not believe that vaccines should be stigmatized simply because they have been developed by the most advanced methods available (see: Exhibit F-4).

As stated in the "Safety Considerations for Commercial and Environmental Applications of Organisms Derived by Recombinant DNA Techniques," drafted by the E.P.A. and presented in Paris at a meeting of the Organization of Economic Cooperation and Development (O.E.C.D.)

Genetic engineering of organisms existed long before the ability to modify directly the cell material; it dates from man's recognition that animals and crop plants could be selected and crossed to produce a desired phenotype. ...Consequently, there is no scientific justification for singling out rDNA-related work for a special regulatory regime over and above that which is already established to regulate traditional biotechnology.

Exhibit F-1**Registrations**

1. Principal Investigator Saul Kit, Ph. D. Dept. Biochemical Virology Ext. 4507
 Primary working Investigator Hamida Qavi, Ph. D.
 Technicians None

2. Agent being used Herpesviruses
☒ (X) INFECTIOUS ☐ () CARCINOGENIC ☐ () TOXIC

3. Location where animals will be housed Vivarium (4th Floor)

4. Brief Protocol of Project: To assess the comparative virulence or avirulence of related viruses, wild-type strains, and thymidine kinase-negative mutants of herpesvirus

- a) Route of Exposure of Animal to Agent or Compound Mice --/Will be inoculated subcutaneous or intracerebrally with viruses and observed for about 21 days
 b) Known Routes of excretion or shedding of Agent or Compound
 Not applicable (NA)
 c) Effect of Agent or Compound on animal
 In rabbits, virus will provoke immunological response. Infection with virulent strains will be lethal at high doses to mice.
 5. Precautions necessary to protect animal care personnel and animals in area
 Animals will be handled only by personnel of Biochemical Virology

6. Methods of Disposal

- (a) paper waste - NA
 (b) liquid waste - NA
 (c) animal waste and carcasses - Standard vivarium procedures
 (d) air - NA

7. Recommended guidelines from the literature or granting agencies
 NA

8. Effect of agent or compound on human including system involved and symptoms.

NA

Note: SPECIES OF EXPERIMENTAL ANIMAL
 MUST BE DEFINED - Mice and rabbits

Saul Kit
 PRINCIPAL INVESTIGATOR
 Saul Kit, Ph. D.
Hamida Qavi
 BIOHAZARD CONSULTANT

RECOMMENDATION:

☐ () DISAPPROVED ☒ (X) APPROVED

Signed Samuel Kit 20 Sept. 1983
 Chairman, Vivarium Biosafety Committee

James E. Howell M.D. / 10/1/83
 BIOHAZARD SAFETY COMMITTEE CHAIRMAN

REQUEST FOR USE OF LABORATORY ANIMAL MEDICINE FACILITIES

The purpose of this form is to indicate to the granting agency and/or investigator that the Division of Laboratory Animal Medicine can provide the animal resource and care support for the proposed project, as required by Federal, State, and local laws, regulations, and guidelines.

INSTRUCTIONS: Complete and submit in triplicate for new, renewal, and continuation grant applications or any other proposed use of the facilities. The original must be submitted with the grant application to the responsible institutional administrative authority.

Structure and Function of the S Components of Attenuated and Virulent Pseudorabies Virus Strains

- Title of proposed project: Structure and Function of the S Components of Attenuated and Virulent Pseudorabies Virus Strains
- Granting agency or source of funds: National Science Foundation
- Duration of project: from April 1, 1984 to March 31, 1987
- Proposed location: ☒ BCM ☐ Fondren-Brown ☐ St. Luke's Tower
☐ Wynne Unit ☐ Neurosensory ☐ Other _____
- Proposed animal purchase, maintenance, and care:

animal species ¹	total number of animals for project	funds allocated for animal purchase	funds allocated for animal boarding & misc. animal care ²	duration each animal is to be held
Mice	500	\$500	\$500	14-21 days
Rabbits	4	\$100	\$760	6 mos - 1 yr

¹ If dogs or cats, specify conditioned or nonconditioned.

² In addition to boarding costs, it is recommended that allowance be made for diagnostic laboratory fees, medication charges, etc.

- Indicate special requirements, if any, and explain.

☐ housing ☐ water ☐ light cycle
☐ diet ☐ temperature ☐ cleaning

Explanation: Standard

- Are any of the following to be utilized? If so, attach appropriate approval forms.

☐ recombinant DNA ☒ infectious or potentially
☐ toxic chemicals and/or materials infectious agents to humans
☐ radionuclides and/or animals

- Project personnel: list names and office phone numbers of

Principal Investigator Saul Kit, Ph. D. (713) 799-4507
Primary Working Investigator Hamida Qavi, Ph. D. (713) 799-4507
Technicians _____

9. Briefly explain all experimental procedures to be performed on animals, or attach a copy of that portion of the grant application which contains this information.
 Mice will be inoculated intracerebrally or subdermally with herpesviruses to study comparative virulence of virus strain.
 Rabbits will be immunized by subcutaneous injection and bled from the ear or cardiac puncture to obtain antibodies.

10. The anesthetic, analgesic, or tranquilizing drug to be used on animals during the project to relieve unnecessary pain and distress will be:

drug ketamine
 route IM
 dosage 44 mg/Kg

11. Will the project involve pain or distress to animals without the use of an appropriate anesthetic, analgesic, or tranquilizing drug? IF YES, state why it would be inappropriate to use drugs. (Injections and blood sampling need not be described.)

No

12. Describe post-surgical care to be provided for animals during project.

No

13. Indicate drug and/or procedure to be used to euthanize animals at conclusion of project.

drug _____
 route _____
 dosage _____

or procedure animals will be killed by using CO₂ or by cervical fracture

14. I certify that the use of all animals involved in this project will be carried out within the provisions of Public Law 89-544 as amended by the Animal Welfare Act of 1970 (Public Law 91-579), DHEW publication NIH 73-23 "Guide for the Care and Use of Laboratory Animals" revised 1972, NIH Animal Welfare Policy HEN TN 73.2 (5-14-73), and related animal welfare rules and regulations henceforth issued by the Secretary of Agriculture and/or other Federal or State agencies.

Saul Kit
 signature of Principal Investigator
 ("per" signature not accepted)
 Saul Kit, Ph. D.

Biochemical Virology
 department

9/20/83
 date

APPROVED:

(X) Management	signature <u>Bob L. Anderson</u>	date <u>9-20-83</u>
(X) Veterinarian	signature <u>E. M. Dwyer, DVM</u>	date <u>9/20/83</u>
() Animal Resources Committee (if necessary)	signature _____	date _____

REMARKS:

BAYLOR COLLEGE OF MEDICINE
 REGISTRATION OF P-1 EXPERIMENTATION
 INVOLVING RECOMBINANT DNA

GRANT NUMBER National Science Fdn.

PROJECT TITLE STRUCTURE AND FUNCTION OF THE S COMPONENTS OF ATTENUATED AND VIRULENT PSEUDORABIES VIRUS STRAINS

A. DESCRIPTION

Pseudorabies virus DNA fragments will be cloned in prokaryotic plasmids, such as pBR322, subcloned in phage M13, and sequenced. Cloned DNA fragments will be used in marker rescue experiments.

B. ASSESSMENT OF LEVELS OF PHYSICAL AND BIOLOGICAL CONTAINMENT

Pl, EKI (Federal Register 45, 6732, Paragraph III O, Jan. 29, 1980)

- C. I agree to accept responsibility for training of all laboratory workers involved in the project. I agree to comply with the NIH Requirements pertaining to shipment and transfer of recombinant DNA materials. I am familiar with and agree to abide by the provisions of the current NIH Guidelines and other specific NIH instructions pertaining to the proposed project. The information above is accurate and complete.

Saul Kit
 Principal Investigator
 Saul Kit, Ph. D.

(713) 799-4507

Telephone No.

Sept. 20, 1983
 Date

The above project is on file with the BioSafety Office of the
 Recombinant DNA Subcommittee for approval.

Billy C. Perkins
 Billy C. Perkins
 Biological Safety Officer

Sept. 20, 1983
 Date

REQUEST FOR USE OF BIOHAZARDOUS AGENTS IN ANIMALS

1. Principal Investigator SAUL KIT, Ph. D. Dept. Biochemical Virology Ext. 450
 Primary working Investigator Drs. D. Trakula, M. Otsuka, & D. R. Dobbs
 Technicians None
2. Agent being used Herpesviruses, vaccinia virus
☒ (x) INFECTIOUS ☐ () CARCINOGENIC ☐ () TOXIC
3. Location where animals will be housed Vivarium (4th Floor)
4. Brief Protocol of Project: To assess the comparative virulence or avirulence of related viruses, wild-type strains, and thymidine kinase-negative mutants of herpesvirus and vaccinia viruses will be inoculated intradermally in rabbits and mice and intracerebrally in mice. In addition, in vitro constructed recombinant viruses will be studied.
 a) Route of Exposure of Animal to Agent or Compound animals will be bled to assess immunological response to virus
Intradermally & intracerebrally
 b) Known Routes of excretion or shedding of Agent or Compound infections.
Not applicable (NA)
 c) Effect of Agent or Compound on animal
Virus will provoke immunological response. Infection with virulent strains will be lethal at high doses.
5. Precautions necessary to protect animal care personnel and animals in area
Standard
6. Methods of Disposal
 (a) paper waste - NA
 (b) liquid waste - NA
 (c) animal waste and carcasses - Standard vivarium procedures
 (d) air - NA
7. Recommended guidelines from the literature or granting agencies
NA
8. Effect of agent or compound on human including system involved and symptoms.
None

RECOMMENDATION:

☐ () DISAPPROVED ☒ (x) APPROVEDSigned Irving Rosinson
 Chairman, Vivarium Biosafety CommitteeSaul Kit
 PRINCIPAL INVESTIGATORJ. B. Bredt
 BIOHAZARD CONSULTANTJ. B. Bredt
 BIOHAZARD SAFETY COMMITTEE CHAIR

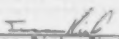
*assuming that the
 recombinant viruses
 referred to above
 do not require
 special containment*

Baylor College of Medicine
BIO SAFETY - RADIATION SAFETY - 713 796-4799DATE October 21, 1983TO: DR. SAUL KITPROJECT TITLE: BIOCHEMICAL ASPECTS OF VIRAL CARCINOGENESISYour request to use HERPES VIRUSES

, a biohazardous agent in animals, has been approved by the Vivarium Subcommittee. The conditions listed in your application and those listed below should be followed without any changes in protocol. Animals involved in this biohazard protocol must be specifically identified according to instructions provided by the Baylor Animal Program.

To assure continuing compliance with BioSafety policy, you will be contacted annually to maintain an updated approval. In addition, changes in any of the following conditions require notification of the Vivarium Subcommittee:

- a. ☒ Any change in protocol
- b. ☐ Any change in area or room
- c. ☒ Unexpected problems or unusual illness in personnel
- d. ☐ Other


Susan Rich, Ph.D.
Chairman
Vivarium Subcommittee

SPECIAL CONDITIONS:

VIVARIUM #10

TEXAS MEDICAL CENTER • HOUSTON, TEXAS 77030

BAYLOR COLLEGE OF MEDICINE

REGISTRATION OF P-1 EXPERIMENTATION
INVOLVING RECOMBINANT DNAUPDATE OF DIVISION OF BIOCHEMICAL
~~SCIENCE~~ VIROLOGY PROJECTSPROJECT TITLE Cloning, Subcloning, Sequencing, and Expression of Viral Thymidine
Kinase (tk) GenesA. DESCRIPTION

Viral DNAs will be isolated, cleaved with restriction nucleases, cloned in pBR322, or in other *E. coli* plasmids, such as pKK223-3, pUC18, and pUC19, subcloned in phage M13mp18 or M13mp19 or related plasmids, or subcloned in phage pSP64 and pSP65. Cloning of portions of the viral DNAs will also be carried out in expression vectors such as pCM-1 CAT, pMUR-2, pMUR-48, or pM1348.

SEE CONTINUATION PAGE

B. ASSESSMENT OF LEVELS OF PHYSICAL AND BIOLOGICAL CONTAINMENT

Pl, HK1 (Federal Register 45, 6732, Paragraph III O, January 29, 1980)

- C. I agree to accept responsibility for training of all laboratory workers involved in the project. I agree to comply with the NIH Requirements pertaining to shipment and transfer of recombinant DNA materials. I am familiar with and agree to abide by the provisions of the current NIH Guidelines and other specific NIH instructions pertaining to the proposed project. The information above is accurate and complete.

Saul Kit (713) 799-4507 12/10/85
Principal Investigator Telephone No. Date
Saul Kit, Ph. D.

See DNA No. 47 dated 10/8/85 and preceding registrations.

The above project is on file with the BioSafety Office of the
Recombinant DNA Subcommittee for approval.

Billy C. Perkins
Billy C. Perkins
Biological Safety Officer

12-13-85
Date

A. DESCRIPTION (continued)

The appropriate tk gene fragments will be sequenced by the dideoxynucleotide chain termination method. Open reading frames, transcription signals, and polyadenylation signals will be identified. Northern blot, hybrid selection, in vitro transcription, and in vitro translation studies will be performed to characterize the tk gene mRNAs and polypeptides.

Herpesvirus and vaccinia virus tk genes will be studied. The herpesviruses to be used include HSV-1 and HSV-2 isolates, marmoset herpesvirus, pseudorabies virus, bovine herpesviruses types 1-6, porcine cytomegalo/lymphotropic isolates, equine herpesvirus type 1, subtypes 1 and 2; equine cytomegaloviruses, equine herpesvirus type 3, herpesvirus sylvilagus, cottontail herpesvirus, feline herpesvirus type 1, and canine herpesvirus.

tk gene expression will also be studied through biochemical transformation experiments, transient expression assays, and marker transfer experiments. To elucidate structural gene and signal sequences, hybrid plasmids will be made consisting of the putative structural portion of one gene and the known signal sequences of another (e.g., HSV-1 tk BglII to BamHI upstream sequences or immediate-early gene signal sequences). The boundaries of the tk genes will be delineated through deletion mutation and insertional mutation of the fragments cloned and subcloned in plasmids and phage. Marker transfer experiments will be used to restore tk⁺ gene function to tk⁻ virus mutants and to prepare tk⁻ deletion mutants of selected viruses.

Exhibit F-2

49 Federal Register 46269 (1984)

III-B-4-a. DNA from any source except for greater than two-thirds of a eukaryotic viral genome may be transferred to any non-human vertebrate organism and propagated under conditions of physical containment comparable to BL1 and appropriate to the organism under study (2). It is important that the fraction of the viral genome being utilized does not lead to productive infection. A USDA permit is required for work with Class 5 agents. (18, 20)

III-B-4-b. For all experiments involving whole animals and plants and not covered by Section III-B-4-a, the appropriate containment will be determined by the IBC (22).

III-B-5. Experiments Involving More Than 10 Liters of Culture. The appropriate containment will be decided by the IBC. Where appropriate, Appendix K, Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules, should be used.

III-C. Experiments that Require IBC Notice Simultaneously with Initiation of Experiments. Experiments not included in Sections III-A, III-B, III-D, and subsections of these sections are to be considered in Section III-C. All such experiments can be carried out at BL1 containment. For experiments in this category, a registration document as described in Section III-B must be dated and signed by the investigator and filed with the local IBC at the time of initiation of the experiment. The IBC shall review all such proposals, but IBC review prior to initiation of the experiment is not required. (The reader should refer to the policy statement in the first two paragraphs of Section IV-A.)

For example, experiments in which all components derive from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III-C and can be carried out at BL1 containment.

Caution: Experiments Involving Formation of Recombinant DNA Molecules Containing no more than Two-Thirds of the Genome of any Eukaryotic Virus. Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single family (17) being considered identical (19)) may be propagated and maintained in cells in tissue culture using BL1 containment.

For such experiments, it must be shown that the cells lack helper virus for the specific families of defective viruses being used. If helper virus is present, procedures specified under Section II-B-3 should be used. The DNA may contain fragments of the genome of viruses from

more than one family but each fragment must be less than two-thirds of a genome.

III-D. Exempt Experiments. The following recombinant DNA molecules are exempt from these Guidelines and no registration with the IBC is necessary:

III-D-1. Those that are not in organisms or viruses.

III-D-2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source though one or more of the segments may be a synthetic equivalent.

III-D-3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species) or when transferred to another host by well established physiological means; also, those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

III-D-4. Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c)). Certain classes are exempt as of publication of these revised Guidelines. This list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 3B10, Bethesda, Maryland 20205.

III-D-5. Other classes of recombinant DNA molecules if the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c)). Certain classes are exempt as of publication of these revised Guidelines. The list is in Appendix C. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 3B10, Bethesda, Maryland 20205.

IV. Roles and Responsibilities

IV-A. Policy. Safety in activities involving recombinant DNA depends on the individual conducting them. The Guidelines cannot anticipate every possible situation. Motivation and good

judgment are the key essentials to protection of health and the environment.

The Guidelines are intended to help the Institution, Institutional Biosafety Committee (IBC), Biological Safety Officer (BSO), and Principle Investigator (PI) determine the safeguards that should be implemented. These Guidelines will never be complete or final, since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the Institution and those associated with it to adhere to the intent of the Guidelines as well as to their specifics.

Each Institution (and the IBC acting on its behalf) is responsible for ensuring that recombinant DNA activities comply with the Guidelines. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level.

The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

IV-B. Responsibilities of the Institution.

IV-B-1. General Information. Each Institution conducting or sponsoring recombinant DNA research covered by these Guidelines is responsible for ensuring that the research is carried out in full conformity with the provisions of the Guidelines. In order to fulfill this responsibility, the Institution shall:

IV-B-1-a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the Guidelines. The Institution as part of its general responsibilities for implementing the Guidelines may establish additional procedures as necessary to govern the Institution and its components in the discharge of its responsibilities under the Guidelines. This may include: (i) Statements formulated by the Institution for general implementation of the Guidelines, and (ii) whatever additional precautionary steps the Institution may deem appropriate.

IV-B-1-b. Establish an IBC that meets the requirements set forth in Section IV-B-2 and carries out the functions detailed in Section IV-B-3.

IV-B-1-c. If the Institution is engaged in recombinant DNA research at the BL1 or BL2 containment level, appoint a BSO who shall be a member of the IBC

Exhibit F-3

Veterinary Services Memorandum No. 800.68,
dated December 4, 1984



United States
Department of
Agriculture

Animal and
Plant Health
Inspection Service

Washington, DC
20250

December 4, 1984

VETERINARY SERVICES MEMORANDUM NO. 800.68

Subject: New Biotechnology for Preparation of Animal Biological Products

To : Biologics Licensees and Applicants
National Veterinary Services Laboratories
Regional Directors
Biologics Specialists
Area Veterinarians in Charge

I. PURPOSE

The purpose is to establish policies and procedures for use of new biotechnological procedures for preparation of veterinary biological products subject to licensure under the provisions of Title 9, Code of Federal Regulations (9 CFR), Parts 101 to 117.

II. POLICIES AND PROCEDURES

A. General

Veterinary biological products prepared using new biotechnological procedures such as recombinant DNA, chemical synthesis, or hybridoma technology will be treated as analogous to products prepared by conventional techniques. A separate license will be required for each such product even if the product is identical in molecular or chemical structure to a naturally occurring substance or a conventionally prepared product.

The unlimited number and kinds of products that may result from these new biotechnological procedures make it impossible to define all requirements in specific terms. Each product must be evaluated individually to determine what will be necessary to establish its purity, safety, potency, and efficacy. Special assays, preferably using *in vitro* methods, may be required for potency and stability determinations. Additional tests may be required to assure safety, especially when live microorganisms are present in the biological product.

3. Recombinant Products

1. Guidelines

The United States Department of Agriculture (USDA) requires recipients of research grants and manufacturers of veterinary biological products using recombinant DNA technology to follow guidelines provided by the National Institutes of Health (NIH). These guidelines are also appropriate for development and production of animal biological products.

"Guidelines for Research Involving Recombinant DNA Molecules" and "Recombinant DNA Research; Physical Containment Recommendations for Large-Scale Use of Organisms Containing Recombinant DNA Molecules" have been published and are periodically updated in the Federal Register. These two documents, along with "Administrative Practices Supplement to the NIH Guidelines for Research Involving Recombinant DNA Molecules" published by NIH, outline the current policy of NIH concerning recombinant DNA research and the procedures required to contain recombinant microorganisms when used in large-scale production. Copies of these documents may be obtained upon request from the Office of Recombinant DNA Activities, NIH, Room 4A52, Building 31, Bethesda, MD 20205.

2. Recombinant DNA Technology

The technology encompasses the isolation, characterization, and insertion of foreign DNA into vectors for the production of foreign gene products in suitable expression systems.

a. Foreign DNA

The genetic information coding for the product of interest is referred to as foreign DNA. It consists of a naturally occurring or chemically synthesized nucleotide sequence known to code for a functional gene product. The specific cloned nucleotide segment coding for the desired product must be defined in data supporting each license application. These data must also include a description of antigen or DNA source material, the nucleotide sequence, and the restriction endonuclease digestion map.

b. Vectors

A vector is a cloning vehicle which provides a suitable origin of replication necessary for production of foreign DNA. Such replicons include plasmids, bacteriophage, or viruses such as vaccinia, bovine papillomavirus, adenoviruses, or SV40. A restriction endonuclease map of the vector construct describing structural genes, regulatory or promoter regions, insert orientation, and a description of readily detectable phenotypic traits on host cells will be required as supporting data.

c. Expression Systems

Production of functional gene products depends on the efficient expression of cloned DNA-vector complexes in suitable host microorganisms such as Escherichia coli, Bacillus subtilis, or Saccharomyces cerevisiae. Tissue culture cells may also be used as expression systems for replication of vectors. The mechanism of transfer, copy number, and the physical state of the constructed vector inside the host cell, integrated or extrachromosomal, should be described.

3. Chemically Synthesized Antigens

When the product consists of chemically synthesized polypeptides, the appropriate amino acid sequences will mimic the antigenic site or epitope found in the native antigen. Supporting data shall include type, degree, and persistence of the immune response following administration of the synthetic peptide. Procedures used to increase and prolong the antibody response such as coupling to carrier proteins or addition of adjuvants, must also be described.

4. Master Seeds

a. Recombinant derived bacterial or viral seed stocks used to prepare veterinary biological products must meet established procedures used to certify Master Seeds for conventionally prepared biological products (9 CFR 101.7).

b. Tissue culture-propagated cells from vertebrate animals used for vector propagation and antigen production must meet the requirements of 9 CFR 113.51 or 113.52.

c. If the gene product in any preparation has been adequately characterized, use of newly constructed Master Seed vectors may be permitted under abbreviated immunogenicity test procedures, subject to reevaluation for safety.

5. Product and Serial Release

Each Outline of Production shall be prepared in accordance with 9 CFR 114.9. Outlines must include procedures to ensure consistency in production and recovery of specific antigenic material. Recovery procedures must include the removal of excessive antibiotic levels (9 CFR 114.10) and undesirable fermentation byproducts such as bacterial endotoxins.

In addition to the serial release tests for purity, safety, and potency, additional antigenic characterization such as electrophoretic mobility or immunoelectrophoresis may be required to demonstrate consistent gene expression.

VS Memorandum No. 800.68

4

C. Monoclonal Antibody Products

1. The specificity and potency of monoclonal antibody products will be compared with that of similar polyclonal antibody products. The specificity and sensitivity of monoclonal antibody products must be at least equal to that of antibody products of traditional polyclonal nature.

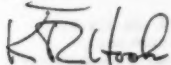
2. Monoclonal antibody products must be derived from Master Cell Stocks which meet the requirements of 9 CFR 113.52. Description of cell cloning procedures and preparation and characterization of cell passages must also be provided.

3. The Outline of Production must provide a description of all processes including scale-up, ascites fluid or cell culture supernate preparation, purification, concentration, and inactivation. Mouse colonies must be screened to demonstrate freedom from adventitious agents, especially those detected by the mouse antibody production (MAP) test. If the MAP test discloses the presence of murine leukemia viruses or other adventitious agents, the product shall not be released unless inactivation procedures approved by Veterinary Services have been conducted.

The immunologic specificity of the monoclonal antibody product must be fully described, and methods used to characterize the product and to evaluate all production serials prior to release must be included in the filed Outline of Production.

III. ENVIRONMENTAL CONSIDERATIONS

Under the present NIH guidelines, the deliberate release of any organism containing recombinant DNA into the environment is subject to review and approval by appropriate Federal agencies. In normal husbandry and laboratory practices, veterinary biological products are not considered to be released into the environment. In the event that a veterinary biological product would be considered to be released into the environment, the issuance of a license or import permit may require an environmental impact statement and interagency approval.



K. R. Hook
Acting Deputy Administrator
Veterinary Services

Exhibit F-4

"Safety Considerations for Commercial and Environmental Applications of Organisms Derived by Recombinant DNA Techniques," drafted by the E.P.A. and presented in Paris at a meeting of the Organization of Economic Cooperation and Development (O.E.C.D.), which consists of 24 nations; and Biotechnology Law Report 59 (January-February, 1986)

INTRODUCTION

This document presents a discussion of the safety issues associated with industrial, agricultural and environmental applications of organisms derived by recombinant DNA (rDNA) techniques. It reflects the opinions of the OECD group of government experts and is based on the best contemporary scientific information available. The focus is on the scale-up and environmental applications of rDNA organisms and their products because this technology has developed and progressed to the stage of commercialization during the last 10 years. The range of scientific considerations discussed can be used to develop safety policies for rDNA processors and products. They are not guidelines or standards for regulating products of rDNA, but a first step towards eventual international harmonization on these issues.

Biotechnology products and processes have been the subject of long-standing extensive regulatory oversight by national health and safety regulatory bodies world-wide. With the mercurial commercial use of rDNA engineered organisms in biotechnology products and processes, questions arose regarding the capacity of these existing approaches to address any possible incremental risks associated with these new techniques. More specifically: what are these possible incremental risks; are they sufficiently dealt with and how; and what additional regulatory oversight is under consideration?

For the purpose of taking initial steps toward international accord, the promotion of international commerce, and the reduction of national barriers to trade in biotechnology, the OECD Committee for Scientific and Technological Policy set up a group of government experts to: review countries' positions on safety in the use of genetically engineered organisms for commercial, environmental and agricultural applications; identify criteria that have been or may be adopted for monitoring of these organisms or authorization of their use for commercial products; and explore ways for monitoring the future production and use of genetically engineered organisms in industry, agriculture and the environment.

In discussing these issues in the document the group emphasized a number of concepts on which their philosophy about biotechnology regulation is based:

- o Biotechnology is not new. Before 6000 BC the Sumerians and the Babylonians exploited the ability of yeast to make alcohol in the form of beer.
- o Pharmaceutical and agricultural industries developed years ago in part by successfully utilizing biotechnology on a commercial scale, and continue to do so today.
- o These industrial and agricultural applications of biotechnology are currently successfully regulated in many countries and have been for years.

- o Pharmaceutical and agricultural industries have safely and successfully used conventional methods of genetic engineering (e.g., natural selection, chemical or radiation induced mutation, cross breeding, conjugation, transformation) on a commercial scale for decades.
- o Risk techniques are refinements of conventional, more crude, genetic engineering techniques and permit extremely sensitive and meticulous manipulations of genes which result in very specific better-characterized effects.
- o The large-scale "quantum" processes for obtaining products from rDNA-containing genetically engineered organisms are fundamentally the same as those employed by conventional techniques. These processes are based on vast experience in both the pharmaceutical and agricultural industries. Moreover, the safety and commercial viability of rDNA-containing organisms have been demonstrated by industrial-pharmaceutical applications in several OECD countries (e.g., human insulin, measles vaccine, phenylalanine, human growth hormone, hepatitis vaccine, interferon, etc.).

The document which follows has been designed to present rationales for approaches a country can take in dealing with the safety considerations of biotechnology in commercial, agricultural and environmental applications.

In the past 10 years a new dimension was added to biotechnology when scientists discovered biological techniques to recombinant *in vitro* the DNA from different organisms. The first, and best known technique, is recombinant DNA (rDNA). It has been the subject of intense laboratory research and commercial development during the past 10 years and has been shown to be safe when used in the laboratory and in the first commercial applications.

The OECD group chose to focus this document only on the issues relating to those products and processes developed by the most well-described techniques of rDNA, although a broader review might have been selected. Justification for this limited approach is supported by the degree to which the public has been involved and interested in rDNA issues for the past 10 years. In addition, all OECD countries have already adopted policies to deal with rDNA research, so there is some existing rationale on which to assess the safety of commercial, agricultural and environmental uses of this subset of biotechnology.

The following are a number of the concepts the group emphasized in developing their criteria for considering rDNA organisms and their products:

- o "Genetic engineering" of organisms existed long before the ability to modify directly the cell material; it dates from man's recognition that animals and crop plants could be selected and crossed to produce a desired phenotype.

CHAPTER V

CONCLUSIONS & RECOMMENDATIONS

(The information in parenthesis indicates the source of the statement).

1. The application of risk techniques provides new and exciting possibilities in a wide range of industries and can be expected to yield considerable benefits to mankind (OECD, #166).
2. Any potential hazards of industrial use of rDNA organisms are expected to be of the same nature as for other biological agents (OECD, #59).
3. Any potential environmental impacts of environmental or agricultural applications are expected to be of the same nature as for other biological agents (OECD, #64).
4. The conjectural risks of the application to biotechnology of recombinant DNA and other techniques can be assessed and managed with current risk-assessment strategies and control methods (AOP, 2; cf. OECD #169).
5. Consequently, there is no scientific justification for singling out rDNA-related work for a special regulatory regime over and above life, which is already established to regulate traditional biotechnology. (AOP Conclusions and Recommendations Drafting Group, #2; cf. OECD #173).

OECD report, *Biotech 2*, 7-32, 1984.

Chapter I outlines the current and potential applications of recombinant DNA technology. Chapter II discusses general features of safety and risk considerations associated with biotechnology, with special emphasis on commercial, agricultural, and environmental applications of the technology. This chapter also describes a philosophy for scientific consideration of risks in this area, and risk-assessment methods both generally and as applied to biotechnology. Chapter III contains a discussion of how safety and risk considerations might be managed through containment mechanisms in the industrial application of microorganisms, when a risk is identified. Chapter IV discusses scientific considerations for possible safety management for agricultural/environmental applications. Chapter V provides conclusions and recommendations.

[Chapters I to IV omitted.]

6. Assessment of potential risks of organisms for environmental or agricultural applications is less developed than the assessment of potential risks for industrial applications. However, the means for assessing rDNA organisms can be approached by analogy with the existing data base gained from the extensive use of traditionally modified organisms in agriculture and the environment generally. With proper (responsible) incremental assessment during the research and development process, the potential risk to the environment of releasing rDNA organisms should be minimised (OECD, #170).
7. Biotechnology, as employed in numerous industries, is generally regarded as safe. To ensure that future developments occur in an equally safe manner, the implementation of new biotechnological techniques and processes should be monitored for long-term, adverse effects (WHO, #1; OECD #171).
8. In those countries where there exists a legislative framework for the control of occupational and environmental hazards in general, and which can be applied to biotechnology, there is no need for scientific and technical justification for additional restraints on rDNA (OECD, #172).
9. Biotechnology contributes in several ways to the improvement of human health, and the extent of this contribution is expected to increase significantly in the near future. To support this development further, the free exchange of public information in this area between all parts of society, as well as between nations, should be strongly encouraged (WHO, #5; OECD Rec. #8, p. 54).
10. Special efforts should be made to improve public understanding of the various fields of biotechnology, especially with regard to safety (WHO, #8; OECD Rec. #8, p. 54).
11. Harmonisation of approaches to oversight of applications of rDNA and other biotechnological techniques depends on a comprehensive understanding of existing mechanisms. Therefore, an international survey of GDT member nations' mechanisms should be performed.

GDT = European Federation for Biotechnology (Kuentz et al.) document, Appl. Microbiol. Biotechnol. 21, 1-4 (1985).

PART SEVEN

The Second Generation Pseudorabies Virus Vaccine

The Second Generation Pseudorabies Virus Vaccine

Prior to the start of our work on PRV in 1982, the USDA emphasized that swine vaccinated with present vaccines cannot be serologically differentiated from infected swine (see: Exhibit A-4). The lack of a serological marker was a strong deterrent to vaccination because vaccination without such a marker would confuse the serological picture. Therefore, an important aim of our research was to develop a vaccine strain containing a serological marker, i.e., a strain lacking a major antigen that was present in all disease-causing field strains. This was accomplished this year in the development by us of PRV(dlg92/dltk) (also called PRV(dlgC/dltk)). Pigs vaccinated with PRV(dlg92/dltk) do not make antibodies to glycoprotein gIII.

The second generation vaccine, PRV(dlg92/dltk), has been sent to TECHAMERICA GROUP, INC. and the USDA pre-licensing process initiated. With the second generation vaccine, we can carry out vaccination/test programs and eventually eradicate pseudorabies disease from farms in America and worldwide.

What about disease in feral pigs and other wild-animal species? We are already involved in preliminary discussions with Dr. Victor Nettles of the University of Georgia and Dr. E.P.J. Gibbs of the University of Florida on methods that might be employed to protect animals in the wild from fatal diseases through vaccination.

The Florida situation is especially noteworthy (see: Exhibit A-1). The Florida panther is an endangered species. The feral pigs of Florida serves as a food supply for the panther. However, Dr. E.C. Pirtle has just shown that 35% of the feral pigs of Florida are infected with a pseudorabies virus. If the panther hunts the feral pigs and/or feeds on meat from feral swine, the panther may be further endangered through pseudorabies infection. A safe vaccine can protect the endangered panther and reduce pseudorabies disease in the feral pigs. Thus, OMNIVAC-PRV can not only be safely and effectively employed to protect swine from pseudorabies infection but, also an endangered species such as the panther.

PERSONAL QUALIFICATIONS

Name: Saul Kit, Ph. D.
Professor of Biochemistry and Head,
Division of Biochemical Virology
Baylor College of Medicine
Houston, Texas, U. S. A.

Date and Place of Birth: November 25, 1920
Passaic, New Jersey

Marital Status: Married, September 28, 1945; Dorothy Anken
Three children

Honors: Research Career Award, 1963
National Institutes of Allergy and
Infectious Diseases

Education: B. A., University of California, Berkeley, 1948.
Ph. D., University of California, Berkeley, 1951.
Sigma Xi, Phi Beta Kappa, Highest Honors in
Biochemistry with B. A. Degree.

1949 - 1950 Abraham Rosenberg Predoctoral Fellow,
University of California, under the guidance of
Dr. D. M. Greenberg.

1950 - 1951 National Cancer Institute Predoctoral Fellow,
University of California, under the guidance of
Dr. D. M. Greenberg.

1951 - 1952 National Foundation for Infantile Paralysis Postdoctoral Fellow,
University of Chicago, under the guidance of
Dr. E. S. Guzman-Barron.

1952 National Cancer Institute Postdoctoral Fellow,
University of Chicago, under the guidance of
Dr. E. S. Guzman-Barron.

Subjects of Interest: Molecular Biology
Biochemistry of Cancer
Viral Oncogenesis

Professional Qualifications, Page 2

Professional Appointments:

- 1953 - 1955 Research Biochemist, Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.
- 1956 - 1957 Assistant Professor, (Clinical), Department of Biochemistry, Baylor University College of Medicine, Houston, Texas.
- 1956 - 1957 Assistant Biochemist, Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.
- 1957 - 1958 Associate Professor, (Clinical), Department of Biochemistry, Baylor University College of Medicine, Houston, Texas
- 1957 - 1960 Associate Biochemist, Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.
- 1959 - 1961 Associate Professor, Department of Biochemistry, The University of Texas Post Graduate School of Medicine, Houston, Texas.
- 1961 - 1962 Biochemist and Chief, Section of Nucleoprotein Metabolism, Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.
- 1962 Spring Visiting Professor of Virology, Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas.
- 1962 - Present Professor of Biochemistry and Head, Division of Biochemical Virology, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas.
- 1963 - 1986 Research Career Award, National Institute of Allergy and Infectious Diseases.

Memberships in Medical and Scientific Societies:

American Society of Biological Chemists
 American Association for Cancer Research
 American Society for Cell Biology
 American Society for Microbiology
 Argentine Society of Virology (corresponding member)
 American Society for Virology

Mr. VOLKMER. Thank you very much, Dr. Kit.

Dr. Bartkoski, your statement also will be made a part of the record at this time in the record. You may review your statement in full or summarize, however you so desire, Dr. Bartkoski.

Dr. BARTKOSKI. Mr. Chairman and distinguished members of the subcommittee:

My name is Michael J. Bartkoski, Jr., and I am vice president of Biologics Corp., a division of TechAmerica Group.

I am here today to address the subcommittee's concerns regarding the U.S. Department of Agriculture's review and licensing of Omnivac-PRV, a recombinant derived pseudorabies virus vaccine manufactured by TechAmerica Group. I hope my presentation today will alleviate any doubts about the comprehensive procedures TechAmerica followed to obtain a license from the USDA.

TechAmerica is a multifaceted veterinary health products company with major divisions in both veterinary drugs and biologicals and with annual sales of approximately \$30 million. Biologics Corp., a division of TechAmerica, and its predecessor have been in the veterinary biological business for 20 years.

Biologics Corp. has obtained approximately 77 veterinary biological licenses from the USDA during this period. Thus, the company has extensive experience working in this sensitive, but heavily regulated, vital industry. TechAmerica has a historical record, not only for product development, but also for product manufacturing and for commercial distribution.

The procedures that TechAmerica followed to obtain a license for Omnivac-PRV were comprehensive and entailed careful compliance with the USDA regulations and with the NIH guidelines. These regulations work in conjunction to provide guidance for the development of safe and effective products. A two-tiered system thus exists. The NIH guidelines provide the framework for laboratory and preclinical research, though they are binding for only federally funded projects.

For actual product development beyond that stage, and in some cases even before, the binding legal obligations from the Federal regulatory statute apply. This system has functioned well and has led to the current marketing of such genetically engineered products as human insulin, human growth hormone, as well as numerous human and veterinary biological diagnostic tests.

For the currently marketed products, it should be important to recognize that the environmental assessments have been prepared and no significant impact on the environment has been found.

TechAmerica has complied with all of its obligations from the Virus-Serum-Toxin Act, plus the accompanying regulations, the guidelines from the NIH, in a spirit of good faith and in full cooperation. The vaccine satisfied all of the stringent standards of these requirements and was proven to be pure, safe, and potent.

In addition, the Animal, Plant Health Inspection Service of the USDA conducted an environmental assessment of the product which confirmed these findings and concluded that Omnivac-PRV did not have a significant impact on the environment.

The development of Omnivac-PRV has followed the new standard course of product development of genetically engineered products. In light of public concern surrounding genetic engineering

and biotechnology, however, it is important for the subcommittee and its staff to acquire a complete understanding of the nature and development of the recombinant derived pseudorabies virus vaccine and its significance to the improved treatment of pseudorabies virus infection in swine.

This vaccine was developed to counteract the pseudorabies virus. Pseudorabies is a contagious disease of swine but poses no health risk to humans. Pseudorabies virus causes respiratory illness in swine which is often fatal. In addition, the disease often causes abortions, reduced litter size, and reduced growth rates in swine. The excuse me—the effect of pseudorabies in swine is usually fatal in young animals and can devastate the entire herd.

During the last decade, the prevalence of the disease has increased by tenfold, and is responsible for major economic losses to the swine industry.

Vaccines for pseudorabies have been approved for use for many years. Prior to the development of Omnivac-PRV, the available vaccines consisted of modified live or killed vaccines. In modified live vaccines the virus contains all of the genetic information required for self-replication.

The effectiveness of these conventional vaccines is marred by the unique nature of the virus itself, which can establish itself in the animal's central nervous system and become latent for long periods.

During times of stress, however, the virus once again can become active and replicate. Therefore, conventionally vaccinated animals can become carriers of pseudorabies virus and can transmit the virus to other animals. The Omnivac-PRV is a major innovation because its recombinant derived virus apparently cannot multiply in the animal's central nervous system. The vaccine, therefore, is much safer because it cannot establish latency in vaccinated animals.

The Omnivac-PRV vaccine was developed by using recombinant DNA procedures to further modify the existing modified live virus that has been licensed by the USDA as a pseudorabies vaccine.

The procedures used deleted the gene coding for the enzyme thymidine kinase, or TK, from the viral DNA. Without the TK enzyme, the virus appears unable to replicate within the central nervous system. As a result, Omnivac-PRV vaccinated animals cannot serve as a source of infection to the other animals.

The recombinant derived pseudorabies virus vaccine was originally developed by Dr. Saul Kit at the Baylor College of Medicine. Dr. Kit conducted developmental studies in both the laboratory and the field which clearly evaluated the safety features of the recombinant derived vaccine. These studies were conducted prior to TechAmerica's license application, but provided background to the USDA with extensive safety data which were used during the licensing process.

In conclusion, Mr. Chairman and members of the subcommittee, Omnivac-PRV marks an innovation in combating a devastating disease in swine and has been well documented in a safe and effective manner. Just as modified live vaccines represented an improvement over killed vaccines in treating pseudorabies virus; so, too,

does genetically engineered Omnivac represent an improvement in safety and effectiveness over modified-live vaccines.

All vaccines present complex questions of safety. And since the inception of vaccines, the issue of safety has required comprehensive testing and evaluation regardless of whether the source of the vaccine be killed, modified-live, or genetically engineered. The basic regulatory structure is designed to ensure the safety of such products regardless of the technology used in their manufacture.

Thank you very much.

[The prepared statement of Dr. Bartkoski follows:]

STATEMENT OF
MICHAEL J. BARTKOSKI, JR., Ph.D.
VICE PRESIDENT FOR OPERATIONS
BIOLOGICS CORPORATION
(DIVISION OF TECHAMERICA GROUP, INC.,
ANIMAL HEALTH DIVISION)

BEFORE THE
COMMITTEE ON SCIENCE AND TECHNOLOGY
SUBCOMMITTEE ON INVESTIGATIONS AND OVERSIGHT
U.S. HOUSE OF REPRESENTATIVES
APRIL 29, 1986

Mr Chairman and distinguished members of the Subcommittee, my name is Michael J. Bartkoski, Jr. I am Vice President of Operations of Biologics Corporation, a division of TechAmerica Group, Inc. I am here today to address the Subcommittee's concerns regarding the United States Department of Agriculture's ("USDA") review and licensing of Omnivac-PRV, a recombinant derived pseudorabies virus vaccine manufactured by TechAmerica Group, Inc. I hope my presentation today will alleviate any doubts about the comprehensive procedures TechAmerica followed to obtain a license from the USDA.

TechAmerica is a multi-faceted veterinary health products company with major divisions in both veterinary drugs and biologicals with annual sales of approximately \$30 million. Biologics Corporation, a division of TechAmerica, and its predecessor have been in the veterinary biologics business for

20 years. Biologics Corporation has obtained approximately 77 veterinary biologics licenses from the USDA during this period. Thus, the company has extensive experience working in this sensitive, but heavily-regulated, vital industry. TechAmerica has an enviable historical record, not only for product development, but also for product manufacturing and commercial distribution.

A. The Statutory and Regulatory Framework for Licensure of Genetically Derived Products.

The procedures that TechAmerica followed to obtain a license for Omnivac-PRV were comprehensive and entailed careful compliance with the USDA regulations and with the National Institutes of Health (NIH) guidelines. The USDA and NIH guidelines work in conjunction to provide the regulated industry with guidance for the development of safe and effective products in a manner that protects the integrity of the existing statutory regulatory system, in this case, the Virus-Serum-Toxin Act ("VST Act"), without requiring unnecessary interaction with APHIS or NIH to assess every step in the product development process.

In essence, a two-tiered system exists. The NIH guidelines provide the framework for laboratory/pre-clinical research, although they are only binding for Federally-funded

research. For actual product development beyond that stage, and in some cases even before, the binding legal obligations of the applicable Federal regulatory statute apply. This system has functioned well and has led to the current marketing of such genetically-engineered products as insulin and human growth hormone, as well as numerous human in vitro diagnostic agents. For the currently marketed products, it also is important to recognize that environmental assessments have been prepared and no significant impact on the environment has been found.

TechAmerica complied with all of its obligations under the VSF Act, accompanying regulations, and NIH guidelines in a spirit of good faith and full cooperation. The Omnivac-PRV satisfied all of the stringent standards of these requirements and was proven to be pure, safe, potent, and efficacious. In addition, the Animal and Plant Health Inspection Service (APHIS) of the USDA conducted an environmental assessment of the product which confirmed these findings and concluded that Omnivac-PRV did not have a significant impact on the environment.

The development of Omnivac-PRV has followed the now standard course for product development of genetically-engineered products. In light of the public concern surrounding genetic engineering and biotechnology, however, it

is important for the Subcommittee and its staff to acquire a complete understanding of the nature and development of the recombinant derived pseudorabies virus vaccine and its significance to the improved treatment of pseudorabies virus infection in swine.

B. The Pseudorabies Virus Vaccine

Omnivac-PRV was developed to counteract the pseudorabies virus (PRV). PRV is a contagious disease for swine and other livestock, but poses no health risk to humans. PRV causes respiratory illness and encephalitis in swine which is often fatal. In addition, the disease often causes abortions, reduced litter size, and reduced growth rates in swine. The effect of PRV in swine is often fatal to young animals infected and can devastate entire herds.

In the last decade, the prevalence of the disease has increased by tenfold, and is responsible for major economic losses to the swine industry. Moreover, PRV is a particularly gruesome disease which, in some instances, causes animals other than swine to scratch their skin off and amputate their own appendages.

Vaccines for pseudorabies have been approved for use for many years. Prior to the development of the Omnivac-PRV vaccine, the available vaccines consisted of modified live or killed vaccines. In modified live vaccines the virus contains all of the genetic information required for self-replication.

The effectiveness of these conventional vaccines is marred by the unique nature of the virus itself, which can establish itself in the animal's central nervous system and become latent or inapparent for long periods. During times of stress, however, the virus once again can become active and replicate. Therefore, conventionally vaccinated animals can become carriers of PRV if subsequently exposed to the wild-type virus and can transmit the disease to other animals. The Omnivac-PRV is a major innovation because its recombinant derived virus apparently cannot multiply in the animal's central nervous system. The vaccine, therefore, is much safer because it cannot establish latency in infected animals.

The Omnivac-PRV vaccine was developed by using recombinant DNA procedures to further modify an existing modified live virus that has been licensed by USDA as a pseudorabies vaccine. The procedures used deleted the gene coding for the enzyme thymidine kinase (TK) from the viral DNA. Without the TK enzyme, the virus appears unable to replicate within the central nervous system. As a result, Omnivac-PRV vaccinated animals cannot serve as the source of infection to other animals.

The recombinant derived pseudorabies virus vaccine was originally developed by Dr. Saul Kit at the Baylor College School of Medicine. Dr. Kit conducted early developmental

studies in both the laboratory and the field which clearly evaluated the safety features of the recombinant derived vaccine. These studies, which were conducted prior to TechAmerica's license application, provided the USDA with extensive safety data and were used as background information in the licensing process.

In conclusion, Mr. Chairman and members of the Subcommittee, Omnivac-PRV marks an innovation in combatting a devastating disease in swine and other livestock in a well-documented, safe and effective manner. Just as modified live vaccines represented an improvement over killed vaccines in treating PRV, so to does genetically-engineered Omnivac-PRV represent an improvement in safety and effectiveness over modified- live vaccines. All vaccines present complex questions of safety and effectiveness. Since the inception of vaccines, the issue of safety and effectiveness has required comprehensive testing and evaluation regardless of whether the source of the antigen be killed, modified-live, or genetically-engineered. The basic regulatory structure is designed to ensure the safety and efficiency of such products regardless of the technology used in their manufacture.

On behalf of TechAmerica Group, Inc., and myself, I appreciate your providing us the opportunity to explain the development and technology involved in the manufacture of Omnivac-PRV.

Mr. VOLKMER. Thank you very much, Dr. Bartkoski.

Dr. Kit, you have been, even though you may not on this specific—research on this specific DNA application for pseudorabies as herpesvirus vaccine, haven't you received funds from NIH to study the TK gene and herpesvirus in the past?

Dr. KIT. I have been studying herpesvirus and the thymidine kinase gene since roughly 1961. That research was supported by the NIH and the National Science Foundation, the American Cancer Society, and by private contracts.

Mr. VOLKMER. Now, so this you are familiar with NIH procedures and NIH guidelines and ARRC?

Dr. KIT. I have served for 5 years as the chairman of the pathology study section. I have reviewed countless grant applications. I am very familiar with the entire peer review process and the NIH system.

Mr. VOLKMER. Now, so you have agreed in the past, through your procedures, to follow specific NIH instructions, proposed for any recombinant project?

Dr. KIT. Yes; indeed.

Mr. VOLKMER. Now, during the research on the specific project here—where I understand you actually produced two—but only one marketed strain; is that correct? There was two produced, that were TK related?

Dr. KIT. Oh, yes, that's correct. There were two—two vaccines produced which I reported in the first patent. The first is a mutagen induced thymidine kinase negative pseudorabies virus which could serve as a vaccine. And the second is an improvement over the first, since it is a deletion mutant.

Mr. VOLKMER. Right.

Now, as you proceeded with this, did you keep the IBC at Baylor College of Medicine informed of your research?

Dr. KIT. Yes; incidentally, I produced a deletion mutant much earlier for herpes tamarinus, a monkey virus. And the IBC of Baylor has been informed of my work through the years on—ever since actually it was established. I was a member of the ad hoc committee to establish it. I am now a member of the Institutional Biosafety Committee.

Mr. VOLKMER. Now, what is your connection at all with NovaGene?

Dr. KIT. I am the chairman of the Scientific Advisory Board of NovaGene.

Mr. VOLKMER. All right. Does NovaGene have an IBC?

Dr. KIT. No; NovaGene does not have facilities at this time—it does not.

Mr. VOLKMER. All right.

Does it abide by the NIH/RAC guidelines?

Dr. KIT. Very much so.

Mr. VOLKMER. Now, according to the document that you submitted to us, you have performed safety tests, the recombinant derived vaccine as presently being marketed, on mice, rabbits, in the laboratory?

Dr. KIT. Exactly.

Mr. VOLKMER. At Baylor?

Dr. KIT. Yes, yes.

Mr. VOLKMER. And you informed the IBC as those experiments progressed; is that correct?

Dr. KIT. Yes. Specifically, the Biohazard Committee of the vivarium and I informed the vivarium when I requested an assignment for space for the experiments as to exactly what the experiments were.

Mr. VOLKMER. And as I understand, also tests—safety tests—in swine were conducted at the National Animal Disease Center at Ames, IA, in confinement under class 3 containment conditions?

Dr. KIT. That's correct, sir.

Mr. VOLKMER. All right.

Then, in June 1984, the recombinant vaccine was tested during that period—around that period—on approximately 1,400 pigs on the Maddox farm in west central Texas by researchers associated with Baylor, Texas A&M, and the Texas Agricultural Experiment Station.

Were the IBC's at either Baylor or Texas A&M or the NIH/RAC informed of this field test?

Dr. KIT. May I make a correction, sir?

Mr. VOLKMER. Yes; go ahead.

Dr. KIT. I did not perform any of the tests. The tests were performed entirely by the Texas Extension Services under the—under the guidance of the Texas Animal Health Commission. I supplied the vaccine for doing so, and in that way signified my participation in the tests. And, also, I should very strongly emphasize that I do not regard the virus as a recombinant virus according to the definition in the NIH guidelines. Specifically, in the Federal Register, section 3(D)(II) excludes the virus, it is a recombinant derived virus. But it itself is not a recombinant virus.

But the quick answer to your question is that—that I did not inform the Baylor committee, and I did not inform the NIH/RAC Committee of the proposed experiments.

Mr. VOLKMER. All right.

And I would just like to refer you back to a publication that is printed, as I understand, in the High Technology Route to Virus Vaccine published by the American Society for Microbiology in 1985—

Dr. KIT. Yes.

[The material referred to follows:]

Reprinted from HIGH TECHNOLOGY ROUTE TO VIRUS VACCINES
Published by American Society for Microbiology, 1985

Immunization of Pregnant Pigs in a Quarantined Swine Herd with a Thymidine Kinase Deletion Mutant of Pseudorabies Virus

S. KIT,¹ M. KIT,¹ B. LAWHORN,² AND S. MCCONNELL³

Division of Biochemical Virology, Baylor College of Medicine, Houston, Texas 77030,¹ and Texas Agricultural Extension Service² and Department of Veterinary Microbiology and Parasitology,³ Texas A & M University System, College Station, Texas 77843

Aujeszky's disease is an acute and often fatal infection of domestic and feral swine and other animals, caused by a herpesvirus, pseudorabies virus (PRV). Outbreaks of Aujeszky's disease have recently increased in the United States, Europe, Africa, and Asia. Vaccines have previously been used to control Aujeszky's disease but have not prevented circulation of virulent PRV in endemic herds. Test and slaughter programs are now the conventional practice, but their value is suspect as a result of PRV latency in domestic and feral swine. Wild hogs are common in Texas and at least 14 other states of the United States. In a PRV-quarantined Texas swine herd, diseased pigs had coloration indicating that feral boars were their sires; many cross-bred feral pigs identifiable by growth stall-out characteristics were scattered throughout grower-finisher pens. A modified-live virus vaccine, which cannot revert to virulence because of deletion mutations, has been used within this herd to identify previously infected swine and as a barrier to PRV spread. The vaccine virus can easily be distinguished from any other PRV strain. More than 700 grower-finisher pigs, 224 nursery pigs, 128 pregnant females, 56 nonpregnant females, 7 boars, and 224 piglets were immunized. No adverse reactions occurred in females in all stages of pregnancy, boars, or pigs as young as 1 day. Litter sizes were normal. Furthermore, 31 latently infected gilts and animals that may have been exposed to virulent field strains after immunization were identified by anamnestic responses in serum neutralization tests. Laboratory research has also shown that inoculated 5- to 6-week-old pigs were protected against challenge with virulent PRV. Inoculated calves and sheep exhibited no adverse reactions, further attesting to the vaccine's safety and efficacy.

In February of 1984, an outbreak of Aujeszky's disease (AD) occurred in the swine on the Wallace and Tommy Maddox farm in West Central Texas. The possibility was considered that a new experimental vaccine [patent strain PRV (BUK dl3)] that had been developed at the Baylor College of Medicine might be useful for an immunization-eradication program on this farm. Therefore, an experimental inoculation plan was drawn up and implemented with the

cooperation of the Maddox family, the Texas Pork Producer's Association, the Texas Animal Health Commission, the Texas Agricultural Extension Service, and NovaGene, Ltd.

The results of this experiment will be described shortly. But first, it is worthwhile to summarize briefly the clinical and epidemiological aspects of AD, also known as mad itch and infectious bulbar paralysis. The cause of AD is an alpha herpesvirus, pseudorabies virus (PRV; *Herpesvirus suis*). Pigs are the natural host for this virus, and depending on the age of the pig, the infection may result in mortality, morbidity, or latency. However, many other animals are susceptible to PRV (2): this virus causes fatal infections in dogs, cats, cattle, sheep, mink, raccoons, opossums, skunks, rabbits, rats, and mice, although the latter hosts are considered "dead-end" hosts (i.e., the virus dies within the host without passing infection to other animals). Fortunately, clinical disease has never been known to occur in humans exposed to PRV, PRV-infected swine, or pork products.

AD is usually transmitted by acutely infected pigs, which produce large amounts of infectious PRV, or by latently infected animals, which sporadically shed small amounts of virus. Often, PRV is transmitted by pregnant sows to their highly susceptible newborn offspring. Latently infected pigs may also be experimentally induced to excrete PRV by stress-related factors, such as dexamethasone.

With regard to the age dependence of disease signs in PRV-infected swine, young pigs exhibit a high mortality, high morbidity, fever, vomiting, diarrhea, and central nervous system signs. Older (feeder) pigs show a variable mortality, high morbidity, fever, anorexia, listlessness, coughing and sneezing, labored breathing, central nervous system signs, and gastrointestinal signs. In pregnant sows, the mortality is low, but respiratory and gastrointestinal signs are common, as are reproductive problems. Infection sometimes leads to abortion, stillbirths, and mummified fetuses. Of the laboratory animals, rabbits appear to be most susceptible. In our laboratories, we observed that as little as 1 PFU of PRV injected subcutaneously in rabbits induced pruritis, encephalitis, and death in 4 to 7 days.

AD has been recognized in the United States since the mid-1800s. Sporadic outbreaks were often reported prior to 1975, but not until the mid-1970s was AD considered a serious threat to the swine industry. The reasons for the sudden increase in the incidence of AD are not fully understood, but the combined experience in the United States, Europe, Asia, and Africa suggests that the explosion of disease incidence may be related to: (i) changing management practices, particularly intensification of housing; (ii) a decreased incidence of hog cholera, leading to increased recognition of AD; and (iii) possibly, emergence of more virulent PRV field strains.

AD has been increasing at an alarming rate. Tests on sera from slaughterhouses in the United States have documented an increase in PRV-positive sera from 0.5% in 1970 to 3.7% in 1978 and to about 9% in 1983. Authenticated outbreaks of AD have occurred in recent years in Ireland, England, Denmark, the Netherlands, France, and Central and Eastern European countries. There also have been serious outbreaks of AD in Ghana, Nigeria, and other African countries. The disease has appeared in Japan. On Taiwan, where there are 18 million people and 6 million pigs, AD is one of the major diseases threatening the food supply.

pages tightly bound

CONTROL MEASURES

Concerns over the increases in AD have inspired strict controls over the movement of swine within and between the states of the United States, and several government-directed control programs: (i) depopulation-repopulation programs, (ii) test and removal programs, (iii) offspring segregation, and (iv) immunization-eradication programs (13).

Depopulation programs have two serious disadvantages: (i) they are disruptive to management, and (ii) purebred and commercial producers may suffer severe economic losses due to loss of valuable blood lines and the disrupted cash flow resulting from dwindling breeding herd inventories. Offspring segregation permits the retention of bloodlines in herds with high PRV seropositives, but requires segregated housing facilities, considerable management expertise, and the expense of considerable serological testing.

Test and removal programs are the prevailing vogue in the United Kingdom and the United States. These programs, however, require extensive and costly serological testing, they are difficult to apply successfully in large, continuous farrowing operations, and they have been unpopular with hog producers. In a United Kingdom study reported in 1983, for example, AD was confirmed in 419 herds, 3,400 farm visits were undertaken, about 100,000 blood samples were collected and tested, and over 350,000 pigs were slaughtered (1). The program was financed by a tax extracted from the swine producers, who were especially unhappy when they learned that there had been a \$15 million cost overrun requiring additional taxation. Despite these heroic measures, 41 outbreaks of AD were confirmed in Great Britain from January to July of 1984.

Test and eradication programs have been thwarted for at least two important reasons. First, PRV can persist in the latent state for long periods of time in the absence of clinical signs of infection (10). Latently infected animals may escape detection, either because they score as false negative when tested or because, in practice, not every animal is tested. Second, feral swine are prevalent throughout the United States and represent a natural reservoir for PRV (4, 16).

FERAL SWINE IN THE UNITED STATES

Feral swine are abundant throughout the 12 southern states and in Texas, California, Hawaii, Puerto Rico, and the Virgin Islands (16). Feral swine used to be imported into Louisiana on a regular basis to maintain and add new blood lines for several of the hunting clubs in the state. Although this practice has been discontinued, the descendants of these imports are found in most parishes throughout Louisiana. Since the introduction of European wild boars to California in 1923, and the subsequent interbreeding with feral domestic swine, the number of wild hogs in that state has increased to an estimated population of 80,000 (4). California hunters take approximately 30,000 wild hogs yearly from private lands and through paid recreational programs. The nationwide annual hunting harvest is estimated to be over 100,000 wild swine and is indicative of their abundance.

Marauding bands of wild pigs have "terrorized" Florida in growing numbers, rooting up gardens, unearthing buried cables, and even making occasional

forays into the Kennedy Space Center. These animals are descendants of domestic pigs turned loose by farmers who sold their land to the government for the space center.

In Texas, feral swine, known as Piney Wood rooters, are common in Walker, Liberty, Polk, Harris, Montgomery, Trinity, and Newton Counties. They are the targets of hog hunts and have also been sold weekly through many East Texas auction markets. The romantic suspicion is that the Piney Wood rooters are descendants of hogs that deserted an expedition of Spanish conquistadores more than 400 years ago. Russian wild boars also were turned loose in East Texas years ago, and their strain undoubtedly runs through some of the rooters. Most, though, can trace their ancestry to domestic stock that were simply turned loose by early settlers and rounded up whenever pork was needed. Generations of fending for themselves have given these hogs traits similar to those of the wild European pigs: long legs, a ridge of long bristles on the neck, straight bushy tail, and shovel-like snout with razor-sharp tusks.

Recent surveys of trapped or hunter-killed wild hogs in California showed them to be 3% seropositive for PRV (4). Tests run in May 1984 on hogs at the first Texas Piney Wood Rooter Association of America breeding stock sale in Liberty County showed that 18% of the feral Piney Wood roter hogs were seropositive for PRV. In view of these numbers, it was apparent that measures alternative to depopulation-repopulation and test-eradication were worth considering to control the AD outbreak on the Maddox farm. Hence, the proposal for an immunization-eradication experiment.

SOURCE OF THE PRV ON THE MADDOX FARM

Three hypotheses were considered to account for the source of the PRV infections on the Maddox farm: (i) importation of latently infected breeding animals several months earlier, (ii) a PRV strain from an infected hog in a New Mexico livestock show that had hopped across Texas, and (iii) periodic contact of the breeding animals on the farm with PRV-infected feral pigs. The third hypothesis seemed the most likely because: (i) a wild boar shot near the sows in heat on the Maddox farm had a PRV antibody titer of 4; (ii) two of four sick pigs that had been submitted to the Texas Diagnostic Laboratory for analyses had dark coloration, indicating that feral boars were their sires; and (iii) about 70 crossbred feral pigs, identifiable by failure to grow past 80 to 140 pounds to the usual market weight of 220 pounds or more, were found to be scattered throughout the 1,000 pigs in grower-finisher pens.

As an aid to identification, restriction nuclease fingerprinting analyses (11) were performed on the DNA of the PRV isolated from the Maddox farm and on DNA isolated from an infected pig from New Mexico. Analyses were also performed on DNA from Aujeszky, Bucharest, Indiana-Funkhauser, and Illinois PRV strains (Fig. 1-3). These analyses demonstrated that the PRV isolate from the Maddox farm was clearly distinguishable from the other PRV strains. For example, the *Kpn*I-D fragment of PRV(MAD) was smaller than that of PRV(AUJ) and PRV(NM), and the *Kpn*I-K fragment of PRV(MAD) was smaller than the *Kpn*I-K fragment of PRV(AUJ) and PRV(BUK). PRV(MAD) differed from PRV(Ind-F) in the size of *Kpn*I-F, -GH, and -J_S/J_L fragments. The PRV(MAD)

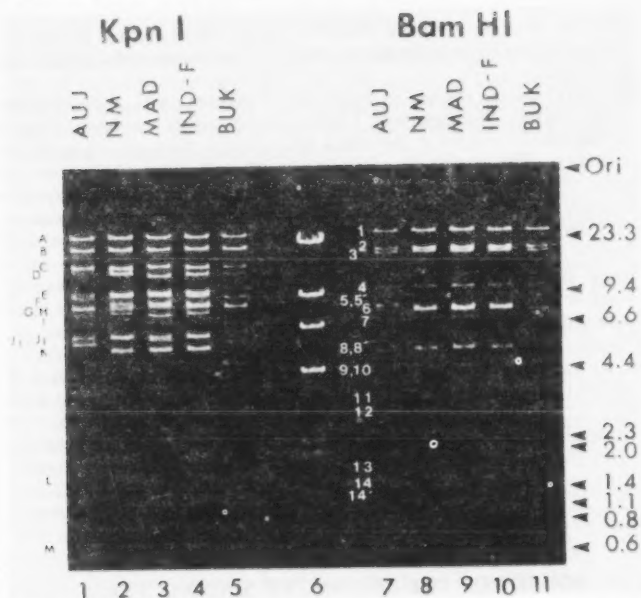


FIG. 1. Agarose gel electrophoresis analyses of DNA from PRV strains after cleavage with the restriction nucleases *Kpn*I and *Bam*HI. The PRV strains shown are Aujeszky (AUJ), New Mexico (NM), Maddox (MAD), Indiana-Funkhauser (IND-F), and Bucharest (BUK). DNA fragments in lane 6 are markers obtained by digesting phage lambda DNA with *Hind*III and phage ϕ X174 DNA with *Hae*III. The letters and numbers identifying the *Kpn*I and *Bam*HI fragments of PRV(AUJ) correspond to the nomenclature for the physical maps of PRV DNA given by Ben Porat et al. (3). Conditions for electrophoresis and staining of DNA fragments with ethidium bromide have been described by Kit et al. (7).

*Bam*HI fragments 9, 10, and 12 likewise distinguished this strain from the other PRV strains (Fig. 1). *Pvu*II and *Sal*I DNA fragments having sizes of about 3 to 7 kilobase pairs provided another characteristic to differentiate the PRV strains (Fig. 2).

The *Kpn*I and *Bam*HI restriction fragments of the Illinois and Aujeszky strains of PRV are shown in Fig. 3. By comparing data of Fig. 3 with those of Fig. 1, it may be concluded that the Illinois and Indiana strains of PRV are very similar and, hence, that the Illinois strain differs in restriction nuclease pattern from the Maddox strain. Finally, it is interesting to note that the size of the *Bam*HI-10 and -12 fragments of PRV(MAD) suggests nucleotide deletions at the inverted repeat (IR)/unique small (U_S) and unique small (U_S)/terminal repeat (TR) regions of PRV(MAD) DNA. Nucleotide deletions in these regions have been

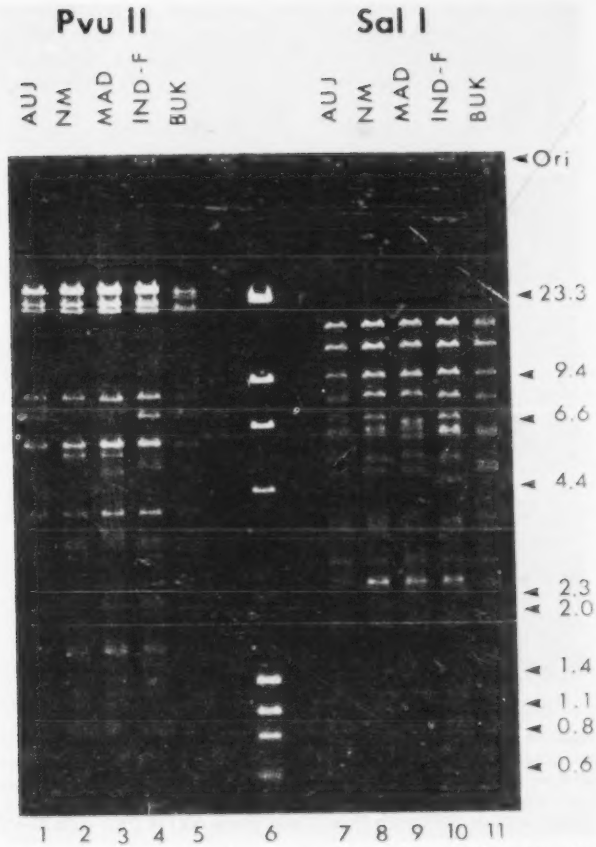


FIG. 2. Agarose gel electrophoresis analyses of DNA from PRV strains after cleavage with restriction nucleases *PvuII* and *SalI*. See Fig. 1 for nomenclature of PRV strains.

observed in our Bucharest vaccine strain [PRV(BUK d13)] and in the Bartha(K) and the Norden(Bucharest) vaccine strains (11).

To provide conclusive evidence that the PRV(MAD) strain had been transmitted by a feral boar, attempts were made by Robert Crandell of the Texas Diagnostic Laboratory to obtain a PRV isolate from a wild pig. Piney Wood rooter hogs with antibodies to PRV were treated with dexamethasone. Nasal swabs and tonsillar scrapes were made, and tissues were explanted or coculti-

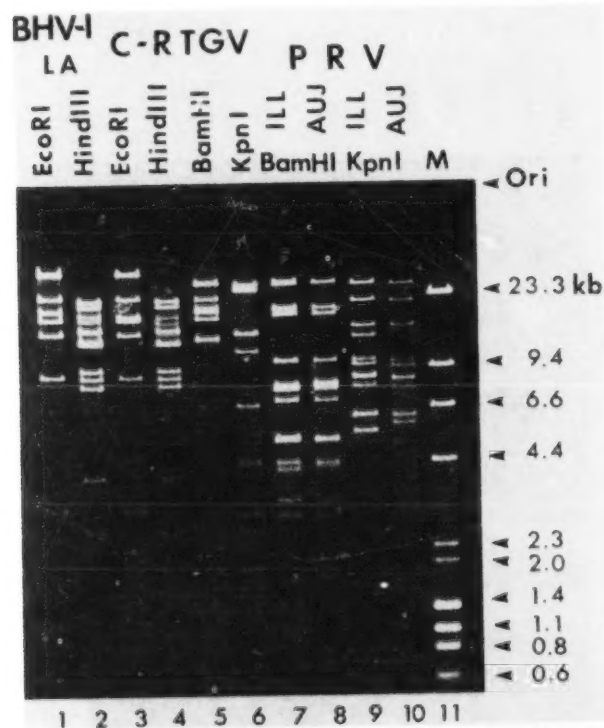


FIG. 3. Agarose gel electrophoresis analyses of restriction nuclease-cleaved DNAs from PRV strains Illinois D1219 (ILL) and Aujeszky (AUJ), from the Los Angeles strain of bovine herpesvirus type 1 (BHV-1 LA), and from the C-RTG virus isolated from dexamethasone-treated Piney Wood rooter hogs. DNA fragments in lane 11 are markers obtained by digesting phage lambda DNA with *HindIII* and phage ϕ X174 DNA with *HaeIII*.

vated, or both, with PRV-susceptible feline kidney cells. A virus, designated C-RTGV, was isolated by cocultivating trigeminal ganglia from one of the seropositive pigs (no. 2515) with feline kidney cells. This virus, however, was not neutralized by known PRV antisera but was neutralized by infectious bovine rhinotracheitis (BHV-1) antisera. Restriction nuclease cleavage fragments of the DNA from the unknown C-RTG virus are shown in Fig. 3. It is obvious that the C-RTGV DNA fragments are quite different from PRV DNA fragments but are similar to the restriction nuclease cleavage fragments of BHV-1(LA) DNA. The results suggest that Piney Wood rooter hogs were latently infected with the

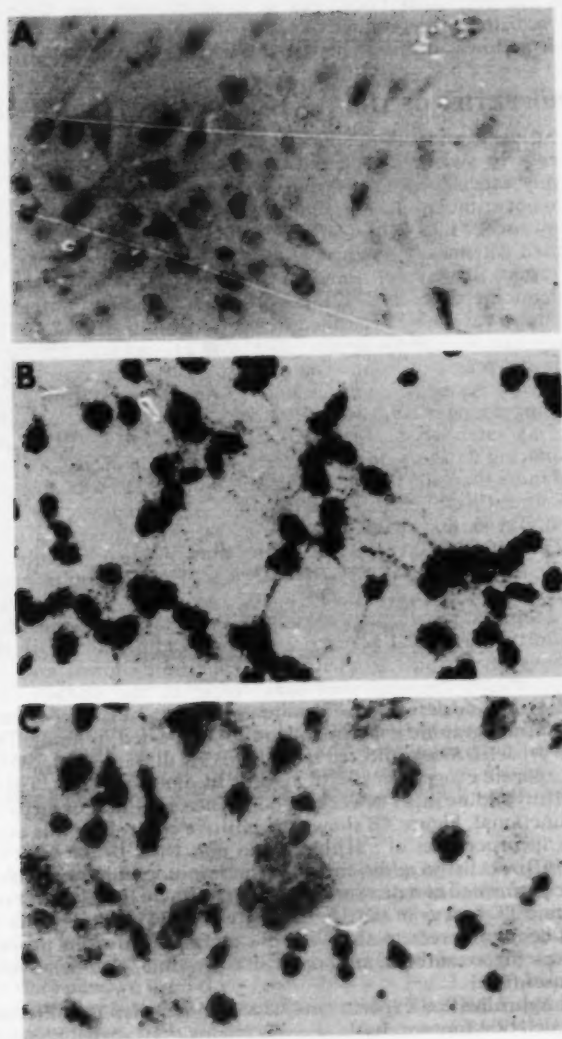
bovine virus. This is interesting because BHV-1 has the capacity to infect swine fetuses and has previously been isolated from stillborn and newborn pigs in herds with a history of reproductive problems (6). In serologic studies, 11% of swine sera from Iowa and Texas herds exhibited positive BHV-1 antibody titers.

PROPERTIES OF THE TK⁻ DELETION MUTANT OF PRV

Inactivated virus and subunit virus vaccines have previously been used to reduce disease in swine herds but have not been very successful; they are comparatively expensive, may be difficult to prepare with sufficient antigen, probably do not stimulate local immunity, and fail to prevent latent infections and recrudescence of PRV field strains. Several modified-live PRV vaccines have also been used extensively in Europe and the United States from 1960 to the present to reduce disease, but they have not prevented circulation of virulent PRV in endemic herds (5, 8, 9, 12). Furthermore, although nonpathogenic for adult pigs, these vaccines have differed considerably in residual virulence for newborn pigs and for calves, lambs, dogs, and rabbits, and they may not be entirely safe for pregnant sows. The transmission of vaccine-induced AD in lambs has been described recently in both Europe and the United States (14).

In developing a new modified-live PRV vaccine, we focused on reducing the virulence of an established vaccine strain, the Bucharest (BUK) strain (12), without impairing its immunogenicity. The isolate chosen for our studies had been passed more than 800 times in chick cells and had previously been studied by Dieter Berger (Washington State University), Jack Howard (University of California at Davis), and Donald Gustafson (Purdue University). The *Bam*HI fragments 7 and 10 of PRV(BUK) are smaller than the corresponding fragments of other PRV strains, and *Bam*HI-12 is completely missing, showing that there are deletions in the U_S region of the PRV(BUK) genome adjacent to the terminal and inverted repeats (Fig. 1). After cloning PRV(BUK), we engineered a deletion mutation into the structural portion of the PRV thymidine kinase (TK) gene. Enzyme assays on extracts from infected cells (not shown) and autoradiographic experiments demonstrated that stocks of the mutant, designated PRV(BUK dl3), had no detectable TK-inducing activity. Figure 4 illustrates, for example, autoradiographic experiments on PRV-infected, mutant, TK⁻ rabbit skin [RAB(BU)] cells. Mock-infected and PRV(BUK dl3)-infected RAB(BU) cells did not incorporate exogenous [³H]thymidine into nuclear DNA. This incorporation of [³H]thymidine into DNA occurs only when a cellular or virus-coded TK activity is functional. Figure 4B shows the positive control for this experiment, namely, the incorporation of [³H]thymidine into DNA by TK⁺ PRV(BUK-5)-infected RAB(BU) cells. In addition, plaque autoradiographic experiments (not shown) were performed and demonstrated that: (i) there is less than 1 in 500,000 plaque-forming TK⁺ virus in the TK⁻ PRV(BUK dl3) stocks, and (ii) TK⁻ PRV(BUK dl3) does not revert to the TK⁺ phenotype even when propagated in HATG medium (hypoxanthine, aminopterin, thymidine, glycine), which selects for TK⁺ viruses (6a).

Molecular hybridization experiments have verified that TK⁻ PRV(BUK dl3) is, indeed, a deletion mutant. In these experiments, ³²P-labeled phage M13mp8 P4 DNA was used as a molecular probe. This phage DNA contains the 148-base



p4 nucleotide sequence that was deleted from the structural region of the PRV TK⁺ gene in generating mutant TK⁻ PRV(BUK dl3). After hybridizing the probe to Southern blots of restriction nuclease-cleaved PRV strains and exposing the blots to X-ray film, it was seen that the probe hybridized to DNA fragments from TK⁺ PRV(AUJ) and TK⁺ PRV(BUK-5) but not to deletion mutant PRV(BUK dl3) (6a). In other experiments, it was shown that a ³²P-labeled DNA probe containing the nucleotide sequences from the entire PRV(BUK-5) TK⁺ gene hybridized both to TK⁻ PRV(BUK dl3) and to TK⁺ PRV(BUK-5) DNA fragments, as expected (data not shown).

SAFETY AND EFFICACY TESTS IN EXPERIMENTAL ANIMALS

Before the PRV(BUK dl3) was used as a vaccine on the Maddox farm, safety and efficacy studies were performed on test animals. First, model experiments were carried out on mice. These experiments showed that parental TK⁺ PRV(BUK-5) had 50% lethal doses (LD₅₀) of about 68 and 2,000 PFU per mouse after intraperitoneal and subcutaneous injections, respectively, into 3- to 4-week-old Swiss mice. In contrast, none of the mice died after intraperitoneal or subcutaneous injections of 4×10^7 PFU of TK⁻ viruses. Inoculated mice were also solidly protected against the lethal effects of 100 to 1,000 LD₅₀ of parental TK⁺ PRV(BUK-5) and the virulent TK⁺ Aujeszky strain of PRV. Experiments utilizing rabbits further demonstrated that the LD₅₀ for rabbits was about 100 to 1,000 times greater for the PRV(BUK dl3) vaccine virus than for parental TK⁺ PRV(BUK-5). These experiments clearly showed that TK⁻ PRV(BUK dl3) was considerably more attenuated than parental TK⁺ PRV(BUK-5) (6a).

Second, safety studies were performed in calves, sheep, and pigs. Four 5- to 7-month-old calves were used for the calf study. In one pen, calf no. 720 was inoculated intranasally with 1 ml per nostril (2.8×10^7 PFU/ml) of TK⁻ PRV(BUK dl3), and contact control calf no. 719 was given the same volume of a diluent placebo. In a second pen, calf no. 718 was injected intramuscularly with 2 ml (2.8×10^8 PFU/ml) of the TK⁻ deletion mutant, while contact control calf no. 712 was injected with diluent fluid. The calves were monitored daily for 10 days for changes in body temperature and for other signs of disease. Nasal swabs were collected on days 1, 3, 5, 7, and 9 after inoculation and assayed for PRV. All four calves were bled for serology on days 0 and 32.

FIG. 4. Autoradiographic experiments showing that TK⁺ PRV(BUK-5)-infected TK⁻ RAB(BU) cells (B) incorporate [³H]thymidine into DNA, but mock-infected (A) and TK⁻ PRV(BUK dl3)-infected RAB(BU) cells (C) do not. RAB(BU) cells were planted on Lab Tech slides at 30,000 cells per well in Eagle minimal essential medium (AutoPow) supplemented with 10% fetal calf serum plus 20 mM bicarbonate plus 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, pH 7.3, plus 2 mM glutamine plus 0.005% neomycin. After 2 days of incubation at 34.5°C, the medium was removed and the cells were infected with 0.1 ml per well of PRV diluted to 10⁴, 10⁵, or 10⁶ PFU/ml. After a 1-h adsorption period, the inoculum was removed, fresh growth medium was added, and the cells were further incubated for 2 h. Then [³H]thymidine (5 µCi and 0.1 µg of thymidine per ml) was added, and incubation was continued at 34.5°C until 20 h postinfection. The slides were rinsed with an isotonic salt solution, fixed with methanol, washed exhaustively with 5% trichloroacetic acid and 100% ethanol, and exposed to X-ray film.

92 CLONING BIOTECHNOLOGY

For virus neutralization tests, 100 TCID₅₀ (50% tissue culture infectious doses) of PRV were used. At a serum dilution of 1:2, all calves were negative on day 0, and calves 712, 719, and 720 were negative on day 32. The intramuscularly inoculated calf no. 718 was, however, seropositive for PRV antibodies at a serum dilution of 1:4 on day 32 after inoculation.

Body temperature remained within normal ranges, and there were no clinical signs of disease in any of the calves. No observable differences were noted between responses of principles and contact controls during the 30-day observation period. Nasal swabs collected and assayed for virus were negative through one blind passage in porcine kidney cells (MVPK) and two blind passages in bovine turbinate (NBTU) cells.

Sixteen yearling sheep that had been weaned on 27 March 1984 were used in the lamb safety study. Each of two yearling Rambouillets was inoculated intramuscularly with 10, 100, or 1,000 PFU of the parental TK⁺ PRV(BUK-5) strain or with 100, 1,000, or 10,000 PFU of TK⁻ mutant PRV(BUK d13). Two uninfected control sheep were kept with each of the virus-inoculated groups. Heparinized blood (buffy coat cocultivation) for virus isolation was collected on days 1, 3, 5, 7, and 9 after inoculation. Serum was collected on days 0 and 31 for antibody assays.

All buffy coat samples were negative for virus through three blind passages. Each lamb was observed daily for signs of clinical disease. No signs of disease were observed, nor were any deviations from normal body temperature noted throughout the observation period. Table 1 shows that control sheep and sheep

TABLE 1. PRV antibody after inoculation of sheep intramuscularly with PRV strains

Sheep no.	Virus inoculated (dose)	Virus neutralization	
		Day 0	Day 31
AB10	PRV(BUK-5) (10 PFU)	-	-
AB11	PRV(BUK-5) (10 PFU)	-	-
AB12	PRV(BUK-5) (100 PFU)	-	-
AB13	PRV(BUK-5) (100 PFU)	-	-
AB14	PRV(BUK-5) (1,000 PFU)	-	-
AB15	PRV(BUK-5) (1,000 PFU)	-	-
AB16	Control	-	-
AB17	Control	-	-
AB18	PRV(BUK d13) (100 PFU)	-	-
AB19	PRV(BUK d13) (100 PFU)	-	-
AB1A	PRV(BUK d13) (1,000 PFU)	-	-
AB1B	PRV(BUK d13) (1,000 PFU)	-	+
AB1C	PRV(BUK d13) (10,000 PFU)	-	+
AB1D	PRV(BUK d13) (10,000 PFU)	-	+
AB1E	Control	-	-
AB1F	Control	-	-

- Negative (-) or positive (+) for PRV antibodies. Serology was carried out at a 1:5 dilution of serum versus 100 TCID₅₀ of PRV. Antibody assays at serum dilutions below 1:5 were not conducted.

inoculated with 10 to 1,000 PFU of TK⁻ PRV(BUK-5) were seronegative for PRV antibodies at 31 days after inoculation. However, one of two sheep inoculated with 1,000 PFU of TK⁻ PRV(BUKdl3) and the two sheep inoculated with 10,000 PFU of the deletion mutant developed PRV antibodies. From these studies, it can be stated that the TK⁻ PRV(BUK dl3) vaccine virus is safe for sheep. Additional studies utilizing even higher virus inocula are planned.

Three pilot studies were performed with pigs. The objective of the first pilot study was to evaluate the safety of the PRV(BUK dl3) vaccine for newborn pigs. A 3-day-old female, a 3-day-old castrated male, and a 5-day-old castrated male pig were used. The 3-day-old pigs were littermates. All pigs were Yorkshire × Duroc × Landrace × Hampshire crosses. All pigs had received colostrum milk from sows obtained from a Qualified, PRV-free herd and parenteral iron dextran (100 mg) before this study. Pigs were weaned from their litters and housed in cardboard boxes, the pair of 3-day-old pigs being housed in one box and the 5-day-old kept in another. Diet consisted mainly of 2 or 3% pasteurized cow's milk fed approximately four times per day. Boxes were kept in a location where the daytime high was estimated to be 100°F (38°C) and the nighttime low was about 75°F (24°C). Pigs were housed in boxes to prevent draft exposure and aid conversion to a substitute diet.

The 5-day-old castrated male pig was inoculated in the neck muscle with 2 ml (10^7 PFU/ml) of PRV(BUK dl3). The 3-day-old female pig was similarly inoculated at the same time, and the castrated male 3-day-old littermate served as a contact control. The rectal temperature of the pigs was followed after inoculation only, since the high environmental temperature and the stress of shipment on the inoculation day caused all pigs to salivate profusely and to breathe rapidly until they cooled down that night. Although the rectal temperature fluctuated, on day 1 after inoculation only the 5-day-old immunized castrate exhibited any elevation in temperature (103.2°F; 39.6°C).

The pigs took about 24 h to switch to the substitute diet. Once they started drinking milk, they never lost their appetites. The 3-day-old pigs experienced a mild diarrhea, possibly from consuming excessive milk. No antibiotics were used in treatment, so if infection was present, it was mild. The three pigs were in good health 18 days after inoculation and were transferred to another premise and switched to solid food.

The second and third pilot studies were performed in collaboration with Eugene Pirtle of the National Animal Disease Center, Ames, Iowa (6a). In the second study, six 5- to 6-week-old Landrace-Hampshire-Yorkshire pigs were inoculated in the gluteal muscle with 1.5 ml (4.7×10^8 PFU/ml) of PRV(BUK dl3). Two other pigs served as controls. Two weeks after inoculation, all pigs were challenged intranasally with 4.3×10^8 TCID₅₀ of the virulent PRV(Ind-F) strain.

One control pig died 4 days after the challenge, and the second was moribund but eventually recovered. Immunized pigs had no signs of illness and were killed 10 and 11 days after challenge. Portions of tonsils and trigeminal ganglia from each immunized pig were ground with sterile sand, triturated in Eagle minimal essential medium containing antibiotics, and inoculated into monolayer cultures of pig kidney cells. No PRV was isolated from the tissues of any of the immunized and challenged pigs.

Before immunization, the sera of the pigs were negative for PRV antibodies. Two weeks after immunization, the pigs had virus neutralization titers of 1:4 to 1:8. After challenge with PRV(Ind-F), the antibody titers increased to 1:256.

The third study involved four groups of 5- to 6-week-old pigs. Group 1 pigs (no. 7 and 8) were vaccinated intramuscularly with 7.5×10^5 PFU of PRV(BUK dl3). Group 2 pigs (no. 5 and 6) were unimmunized controls. On day 21 after immunization, the four pigs in groups 1 and 2 were challenged intranasally with 10^5 TCID₅₀ of the virulent PRV(Ind-F). On day 45, pigs 5, 6, 7, and 8 were bled and given dexamethasone (1 mg/kg) intramuscularly to induce recrudescence of PRV. Dexamethasone treatment continued daily for 5 days. Nasal swabs and tonsil scrapes were obtained from the pigs beginning 2 days after dexamethasone treatment and continuing for 5 days. The pigs were bled for serum 3 to 4 days later and killed, and tissues were obtained for virus isolation.

Group 3 pigs (no. 1 and 3) were inoculated intramuscularly in the same way as group 1 pigs but not challenged. They were bled for serum on day 115 and also treated with dexamethasone, in the same way as group 1 pigs.

Group 4 pigs (no. 2 and 4) were inoculated intranasally rather than intramuscularly and were challenged intranasally on day 29 with 10^7 TCID₅₀ of PRV(Ind-F). These pigs were also later treated with dexamethasone, as in the case of group 1 pigs.

The results of this study may be summarized as follows. Group 2 control pigs (no. 5 and 6) went off feed for 1 day on day 4 after challenge, but group 1 pigs 7 and 8 showed no signs of illness. On day 6 after challenge, PRV was isolated from nasal swabs of group 2 pigs, as expected. After dexamethasone treatment, PRV was not recovered from pigs in groups 1 and 2 from nasal swabs, tonsil scrapes, or from trigeminal ganglia, but PRV was recovered from triturated tonsillar tissues of pigs 6 and 7. The PRV recovered from these two pigs was indistinguishable by restriction endonuclease analysis from the PRV(Ind-F) used to challenge pigs of groups 1 and 2. Pigs of groups 1 and 2 developed virus-neutralizing antibody titers of 64 to 256 at about 45 days after PRV(Ind-F) challenge. Control no. 6 had a fourfold increase in virus-neutralizing antibody titer after dexamethasone treatment, suggesting that an anamnestic response had occurred upon recrudescence of virus.

After dexamethasone treatment, PRV was not recovered from nasal swabs, tonsil scrapes, tonsillar tissue, or trigeminal ganglia of the group 3 pigs that had been immunized only. With regard to group 4 pigs, the vaccine virus was reisolated only on day 3 after inoculation and only from pig no. 4. PRV was also recovered from nasal swabs of group 4 pigs on day 5 after challenge, but only from undiluted swab broth. After dexamethasone treatment, PRV was recovered from nasal swabs and tonsil scrapes of both pigs 2 and 4, and from tonsillar tissue of pig 4 at necropsy. However, PRV was not recovered from trigeminal ganglia.

These observations indicate that the PRV(BUK dl3) vaccine was safe and efficacious in protecting these young pigs from disease signs that would normally have been produced by virulent PRV(Ind-F). Immunization significantly reduced the amount and duration of oropharyngeal shedding after virulent virus challenge (8). It does not appear that the vaccine virus established latency in the trigeminal ganglia of immunized pigs (15), but immunization did

not totally prevent the establishment of a persistent infection in the tonsils by the challenge virus. The results also suggested that TK⁻ PRV(BUK dl3) might be more effective in impeding the spread of virulent virus in herds inoculated intramuscularly than in those inoculated intranasally (8, 9, 12).

IMMUNIZATION OF THE MADDOX HERD

In February of 1984, pigs in six litters on the Maddox farm showed clinical signs of AD, with shaking followed by death in 2 to 3 days. PRV was diagnosed by virus isolation, histopathology, fluorescent antibody, and typical PRV cytopathic effects in tissue culture. Consequently, the Maddox herd was quarantined.

On 24 February 1984, sera from 173 breeding animals were tested. Twelve sows seropositive for PRV antibodies were removed to an isolation pen for slaughter. The litters from the positive sows were separated from adjacent negative-sow litters before and after weaning. In the following 3 weeks, four more litters were affected by AD.

On 26 March 1984, sera testing was repeated. Thirteen breeding sows exhibited PRV antibodies and were removed. A third test was performed on 8 June 1984, but the 185 breeding animals tested were all seronegative. However, the threat of continued exposure from possible PRV-carrier feral swine and the likely circulation of PRV in the 1,000-plus grower-finisher pigs near the "clean" breeding herd made immunization worthwhile.

On 26 and 27 June 1984, approximately 1,275 pigs were inoculated intramuscularly with 2 ml (2×10^7 PFU) of the TK⁻ PRV(BUK dl3) vaccine and ear-notched. About 125 pigs from sows within 3 weeks or less of farrowing at initial immunization were thought to be unprotected by anti-PRV colostral antibody and were inoculated and ear-notched on 18 July 1984. Uninoculated pigs farrowed after 18 July were to serve as sentinels to indicate any subsequent natural PRV spread.

Testing the breeding herd and replacement gilts and boars at 30-day intervals after inoculation was designed to separate infected and noninfected immunized animals and monitor natural PRV spread. As long as any immunized or infected pigs remained on the premise, the herd would remain quarantined.

According to the previous research data, noninfected pigs inoculated once with TK⁻ PRV(BUK dl3) were expected to have virus neutralization titers of 1:8 or less. Pigs incubating or infected with natural PRV were expected to have a significant "booster" rise in titer after immunization. Natural PRV exposure after immunization was also expected to produce a "boosted" titer. On the basis of these expectations, the experiment called for titers of $\leq 1:8$ to be classed as noninfected vaccinates and $\geq 1:16$ to be classed as naturally exposed or infected vaccinates.

On 26 and 27 July 1984, "30-day" blood samples were collected from 211 breeding animals. On 27 and 28 August, "60-day" blood samples were collected from 213 animals. The PRV neutralization titers are presented in Table 2. In the 30-day tests, 189 breeding animals previously seronegative from the 8 June test before immunization had negative antibody titers or titers of 1:2 and 1:4. Twenty-one immunized replacement gilts never before tested and recently

TABLE 2. Antibody formation by pigs on Maddox farm after PRV(BUK dl3) immunization^a

Specimen ^b	Titer ^c		Specimen	Titer		Specimen	Titer		Specimen	Titer	
	30 day	60 day		30 day	60 day		30 day	60 day		30 day	60 day
36	2	2	117	2	N	238	4	N	177	N	2
77	2	N	91	2	N	243	4	2	18	2	2
Tube 4	4	4	88	4	2	60	4	4	13	N	2
	2	N	245	64	D	242	2	N	2	4	2
38	N	4	246	32	D	234	2	NS	8	2	4
84	N	4	247	64	D	180	2	2	92	N	2
48	2	N	248	128	D	175	N	4	51	N	2
71	N	2	249	8	D	224A	4	NS	121	2	N
40	4	2	252	64	D	228	4	2	164	4	NS
72	2	N	253	128	D	230	4	NS	173	N	2
79	4	4	254	64	D	233	4	4	102	2	2
33	4	4	255	32	D	239	2	2	1A	ND	32D
216	2	N	256	64	D	197	N	2	2A	ND	4
217	4	4	257	32	D	196	4	NS	3A	ND	4
204	4	2	258	32	D	187	N	2	4A	ND	N
202	N	2	259	64	D	178A	N	2	5A	ND	16D
205	4	4	260	16	D	22	2	N	6A	ND	16D
73	4	4	261	64	D	192	2	NS	7A	ND	8D
118	2	N	262	64	D	190	N	2	8A	ND	4
210	4	2	263	64	D	195	N	2	9A	ND	2
120	2	N	264	128	D	163	2	N	10A	ND	4
223	4	NS	174A	64	D	57	N	2	11A	ND	2
113	N	2	175A	128	D	29	4	2	12A	ND	4
131	4	NS	176A	32	D	115	4	2	13A	ND	32D
222	2	N	177A	32	D	3	2	N	14A	ND	N
105	2	N	227	4	2	83	4	4	15A	ND	32D
124	2	N	219	4	2	54	2	N	16A	ND	2
212	4	4	215	4	4	32	2	N	17A	ND	4
128	4	2	237	4	4	41	2	2	18A	ND	4
94	2	N	224	2	2	50	2	N	19A	ND	N
220	2	N	214	2	2	179	N	2	20A	ND	4
21	4	4	231	N	2	176	2	2	21A	ND	4
209	2	2	240	4	NS	17	2	2	22A	ND	N
225	2	4	236	4	2	12	4	N			

^a All other animals were negative.^b Specimen number is ear tag number unless specified otherwise.^c N, Negative; NS, no sample; D, gilt moved to isolation pen and sent to slaughter; ND, not done.

selected from the "suspect" finishing pens had titers of 1:16 to 1:128, and one pig (no. 249) had a titer of 1:8. These results suggested that the experimental plan had correctly predicted the titer dividing point between negative vaccinates and infected or exposed vaccinates. Animals with titers of $\geq 1:16$ (and gilt no. 249) were removed to isolation pens and slaughtered.

TABLE 3. Characteristics of litters from sows immunized with TK-PRV(BUK d13)

Description of immunized sows	Total no. of litters	Total no. of pigs born alive	Avg. litter size born alive	Total no. of pigs weaned ^a	Avg. weaned	% (weaned/born alive)	Avg. stillbirths per litter	Avg. mummified per litter	Date of farrowing and location
Control sows: immunized 1 to 2 weeks after farrowing; 95 pigs immunized on same day ^b	12	129	10.75	92	7.66	71.3	NA ^c	0.33	June and July, NPH ^d
Immunized ≤1 to 4 weeks pre-farrowing; two litters immunized at 3 weeks and six not vaccinated	8	91	11.38	73	9.13	80.2	1.25	0	July, NPH
Immunized 34 to 49 days pre-farrowing ^e	17	190	11.18	160	9.41	84.2	0.47	0	July and August, NPH (12), TFH (5)
Immunized 55 to 64 days pre-farrowing ^e	15	143	9.53	135	9.00	94.4	2.26 ^f	0	August through 16 September, TFH
Immunized 68 to 77 days pre-farrowing ^e	19	NR ^g	NA	168	8.84	NA	NA	NA	September, NPH
Immunized 85 to 104 days pre-farrowing ^e	14	148	10.57	129	9.21	87.0	1.21	0	26 September to 10 October, TFH

^a Some piglets lost by trauma or mothers were poor milkers.^b The mortality of two vaccinated pigs was due to exudative epidermitis, and one pig died of small unthrifty pig syndrome.^c NA, Not available.^d NPH, New farrowing house. Wallace Maddox farm; TFH, Tommy Maddox farrowing house.^e Litters not immunized.^f Peaked with height of summer heat.^g NR, Not recorded.

Of the 213 pigs sampled on 27 and 28 August, 58 of 184 sows, gilts, and boars previously immunized had PRV antibody titers of 1:2 or 1:4, including 19 which had scored negative in the July test. Five of 22 replacement gilts never before tested and selected from "suspect" finishing pens had titers of 1:16 to 1:32. All but four of the others were 1:2 to 1:8. The five pigs clearly showing an anamnestic response were also removed and slaughtered. The one gilt with the 1:8 titer was also removed as insurance. The relatively low number of 60-day test gilts with titers of $\geq 1:16$, compared to the 21 gilts selected from the same finishing area having titers of $\geq 1:16$ at 30 days, could be field evidence of vaccine effectiveness.

At the time of the 60-day test, one immunized pig, now 8 weeks old, had a titer of 1:4. This pig's dam had been immunized less than 1 week before farrowing. There were also five 2- to 4-week-old uninoculated pigs from immunized sows that had had 4 to 6 weeks to develop antibodies before they farrowed. None had detectable virus neutralization titers, suggesting that pigs less than 2 weeks old, even coming from immunized sows, can be immunized.

At about 110 days after immunization (14 October 1984), tests were performed on 14 potential replacement gilts that had been immunized on 26 June. However, these gilts were from the finishing pens adjacent to the other gilts having experienced anamnestic responses. Since these gilts had never before been tested serologically, it was decided that the original protocol decision of an antibody titer of $\geq 1:16$ would govern removal of suspect gilts from the breeding program. Five of the 14 samples had titers of 1:16, and the remainder had titers of 1:2 to 1:8. Each of the serum samples was also assayed by the enzyme-linked immunosorbent assay, and all were positive, a good correlation between the virus neutralization and the enzyme-linked immunosorbent assay.

With regard to vaccine safety, there were no identifiable adverse reactions in this herd, even though about 1,400 doses of vaccine were administered. Females in all stages of pregnancy, boars, and pigs as young as 1 day were among the vaccinees. No abortions were observed.

Data depicting the total number of sows that farrowed, the number of pigs born alive and weaned, the average litter size, and the average number of stillbirths and mummified pigs are presented in Table 3. The litter sizes were normal, and immunization did not increase the average number of stillbirths or mummified pigs. No adverse effects have been noted to date on the reproductive capability of the sows.

TABLE 4. Characteristics of newborn pigs immunized with TK⁻ PRV(BUK dl3)

Description of pigs	No. immunized	No. surviving and weaned	Location	Adverse reactions
Immunized at ≤ 1 to 2 weeks of age; from immunized sows	208	204 ^a	TFH (113), ^b NFH (95)	None observed
Immunized at 3 weeks of age; from immunized sows	16	16	NFH	None observed

^a Of the four pigs that did not survive to weaning, two developed exudative epidermitis. One was a very small pig. The reason for the death of the fourth pig was unknown.

^b TFH, Tommy Maddox farrow house; NFH, new farrow house on Wallace Maddox farm.

Data on the pigs immunized at ≤ 1 to 3 weeks of age are shown in Table 4. All but 4 of 224 newborns survived, and no adverse effects attributable to immunization were observed.

In summary, the TK⁻ PRV(BUK dl3) vaccine appears to be safe for newborn pigs, for pregnant sows, and for 5- to 6-week-old pigs, and does not interfere with the interpretations of virus neutralization tests made 30 to 110 days after inoculation. The immunization schedule easily fits into producer's management schemes and creates a potential barrier against natural PRV spread from within a herd or from possible PRV infection from feral pigs outside the herd. Broader testing is indicated, but the present results suggest that the vaccine can inhibit the spread of virulent virus in herds and can be used to eradicate virulent PRV strains from a targeted area.

LITERATURE CITED

1. Anonymous. 1983. Aujeszky's disease. Past, present, and future. *Vet. Rec.* **113**:357-358.
2. Baskerville, A., C. Dow, and J. B. McFerran. 1973. Aujeszky's disease in pigs. *Vet. Bull.* **43**:465-480.
3. Ben-Porat, T., R. A. Veach, and S. Ihara. 1983. Localization of the regions of homology between the genomes of herpes simplex virus, type 1, and pseudorabies virus. *Virology* **127**:194-204.
4. Clark, R. K., D. A. Jeasup, D. W. Hird, R. Ruppanner, and M. E. Meyer. 1983. Serologic survey of California wild hogs for antibodies against selected zoonotic disease agents. *J. Am. Vet. Med. Assoc.* **183**:1248-1251.
5. Donaldson, A. I., R. C. Wardley, S. Martin, and J. W. Harkness. 1984. Influence of vaccination on Aujeszky's disease virus and disease transmission. *Vet. Rec.* **115**:121-124.
6. Joo, H. S., S. A. Dee, T. W. Mollitor, and B. J. Thacker. 1984. *In utero* infection of swine fetuses with infectious bovine rhinotracheitis virus (bovine herpesvirus-1). *Am. J. Vet. Res.* **45**:1924-1927.
- 6a. Klt, S., M. Klt, and E. C. Pirtle. 1985. Attenuated properties of thymidine kinase-negative deletion mutant of pseudorabies virus. *Am. J. Vet. Res.* **46**:1359-1367.
7. Klt, S., H. Otsuka, H. Qavi, D. Trkula, D. R. Dubbs, and M. Hazen. 1980. Biochemical transformation of thymidine kinase (TK)-deficient mouse cells by herpes simplex virus type 1 DNA fragments purified from hybrid plasmids. *Nucleic Acids Res.* **8**:5233-5253.
8. Maes, R. K., C. L. Kanitz, and D. P. Gustafson. 1983. Shedding patterns in swine of virulent and attenuated pseudorabies virus. *Am. J. Vet. Res.* **44**:2083-2086.
9. McCracken, R. M., J. B. McFerran, P. J. McParland, and E. R. McKillop. 1984. Vaccination against Aujeszky's disease. Field experiences. *Vet. Rec.* **115**:348-352.
10. Mock, R. E., R. A. Crandell, and G. M. Mesfin. 1981. Induced latency in pseudorabies vaccinated pigs. *Can. J. Comp. Med.* **45**:56-59.
11. Pirtle, E. C., M. W. Wathen, P. S. Paul, W. L. Mengeling, and J. M. Sacks. 1984. Evaluation of field isolates of pseudorabies (Aujeszky's disease) virus as determined by restriction endonuclease analysis and hybridization. *Am. J. Vet. Res.* **45**:1906-1912.
12. Skoda, R., I. Brauner, E. Sádecký, J. Somogylova, and O. Jamrichova. 1966. Immunization of pigs with BUK vaccine under field conditions. *Vet. Med.* **11**:85-95.
13. Thawley, D. G., D. P. Gustafson, and G. W. Beran. 1982. Procedures for the elimination of pseudorabies virus from herds of swine. *J. Am. Vet. Med. Assoc.* **181**:1513-1518.
14. van Alstine, W. G., T. D. Anderson, P. E. Reed, and J. G. Wheeler. 1984. Vaccine-induced pseudorabies in lambs. *J. Am. Vet. Med. Assoc.* **185**:409-410.
15. van Oirschot, J. T., and A. L. J. Gielkens. 1984. Intranasal vaccination of pigs against pseudorabies: absence of vaccine virus latency and failure to prevent latency of virulent virus. *Am. J. Vet. Res.* **45**:2099-2103.
16. Zygmunt, S. M., V. F. Nettles, E. B. Schotts, W. A. Carmen, and B. O. Blackburn. 1982. Brucellosis in wild swine: a serologic and bacteriologic survey in the southeastern United States and Hawaii. *J. Am. Vet. Med. Assoc.* **181**:1285-1287.

Mr. VOLKMER. With yourself and your son, and Professor Lawhorn and—and Dr. McConnell. And reviewing that, there is no credit given in that publication itself to NovaGene. The credit is given to S. Kit, M. Kit, B. Lawhorn, and S. McConnell, and your division of Biochemical, Virology, Baylor College of Medicine, Houston, TX; M. Kit, Texas Agricultural Extension Service, Department of Veterinary, Microbiology, Texas A&M University, College Station, TX.

Dr. KIT. On page 83 of the reprint, sir, it clearly states: Therefore, an experimental inoculation plan was drawn up and implemented with the cooperation of the Maddox family, the Texas Pork Producers Association, the Texas Animal Health Commission, the Texas Agricultural Extension Service, and NovaGene, Ltd.

Mr. VOLKMER. Correct. But was conducted by the four principals in cooperation—with cooperation of the Maddox family of Texas, et cetera, et cetera.

Dr. KIT. This report is a report of research conducted in a symposium attended by the Department of Agriculture, by industry, and by many scientists, highlighting various approaches of high technology to the development of vaccines. The report includes much information in addition to the specific tests on the Maddox farm. And it is for that reason that the various people are included as authors. There, for example, there are pictures which show the restriction nuclease DNA fingerprints—of the viruses from the farm.

Mr. VOLKMER. Yes; I noticed those.

Dr. KIT. And from other animals. And there is—the evidence indicates that the virus that we are dealing with is a deletion mutant. So this is a scientific report, not merely a report of vaccination.

Mr. VOLKMER. I agree on that. I won't disagree. It is just that I guess it's a question of interpretation, but we will let others make perhaps that interpretation decision rather than this committee—more appropriate bodies.

But there is one area where I think we do disagree and perhaps this hearing will bring this out to where somewhere along the line it is going to have to be—any ambiguity is going to have to be clarified, and that is again on the exemption of experiments and the question of—and I think we disagree with USDA—I see Dr. Bentley over there, we will have him later—on the question of interpretation, releasing into the environment, that question of inoculation of live animals. And, Doctor, let's put it this way—let me put an "if" in here—in the assumption; if we assume that an inoculation into a live animal would be released into the environment, then the field test on the 1,400 would not be an exemption in a field test, would it not; if you assume that?

Dr. KIT. It would still not be recombinant DNA.

Mr. VOLKMER. It is not testing a recombinant product?

Dr. KIT. No, sir. This particular product does not have any DNA from two species mixed together. And, secondly, it was not constructed in the test tube, which is the definition that I have seen for recombinant DNA. This material is made by a normal process which occurs in all cells all of the time, and which for a short hand, we call marker transfer or marker rescue. It is a process that

occurs in cells. It is not a process of splicing in the test tube as defined in a definition of recombinant DNA.

Mr. VOLKMER. But it is a—you don't replace any gene, but you do remove a gene?

Dr. KIT. That's correct.

Mr. VOLKMER. And what you are saying is, when you remove a gene and not replace it with anything else, it is not recombinant DNA?

Dr. KIT. No, no; I haven't said that at all.

I said the recombinant DNA, sir, is defined a very specific way in the guidelines and is defined actually, historically in the original experiment by Berg, Symons & Jackson, which was fusing together a Lambda DNA in the test tube with a Simion virus 40 DNA. It was mixing of two taxonomic species in the test tube to provide an organism that could replicate both in bacteria and in animal cells.

Our virus has nothing to do with this. We have a virus. And as I have indicated in my discussion of the—of the vaccine itself, the vaccine that we started with, that is the Bucharest strain of pseudorabies already has a deletion. And I have indicated in the testimony where that deletion is. That deletion occurred in nature as a normal process. It happened accidentally and it was not known until approximately a year ago. And it was only because of recombinant DNA technology—that is, DNA fingerprinting, and the ancillary techniques such as molecular hybridization, Southern blotting, Northern blotting, sequencing—all of these are recombinant DNA technology.

It was only because of this that we were able to learn that there had been a deletion in the parental Bucharest strains which, incidentally, also occurs in the Bartha Hungarian strain and the NIA4 Irish strain, the other commonly used vaccines.

So all of these products already had deletions which occurred in cells replicating the virus. Our deletion also occurred in a cell replicating the virus and we were able to fish out the deletion mutant that we were looking for by the techniques that I have been using since 1960.

Mr. VOLKMER. Well, it appears, then, there does seem to be some disagreement as to whether or not—or at least in your position, is that this does not fall into the guidelines because basically it is not recombinant DNA procedures that are used to produce a product.

Is that correct? In layman's language?

Dr. KIT. I am attempting to emphasize very clearly my laboratory, since 1979, has been engaged in recombinant DNA technology as understood in the guidelines. That is, in the use of plasmids containing foreign inserts of animal genes, the thymidine kinase gene, and other genes, and other such techniques. And that all of these techniques are important and contribute to amassing the information which makes possible the scientific experiment of marker transfer to give not by splicing, but by normal events in the cell—a deletion mutant that we seek. We can obtain deletion mutants or other mutants by normal processes that occur in a cell, and that have always occurred in the cell.

Mr. VOLKMER. Yes, but not to enhance the deletion of the TK, did not you produce the deletion in the laboratory?

Dr. Krr. We set up the conditions for the deletion to occur by providing millions of tissue culture cells with infectious DNA and the fragment of DNA which contained a thymidine kinase gene suitably altered. And this was allowed to enter the cell through what is called the calcium phosphate technique. Subsequent to this, the infectious DNA initiates replication—millions of progeny particles are produced. And there is a finite possibility that some exchange of DNA fragments will occur in a cell during the mating process and during the normal replication process of the virus in the cell. And from the progeny then, we seek deletion mutants by techniques which permit us to recognize them.

This is not the definition of recombinant DNA technology in the Federal guidelines—excuse me, of a recombinant DNA molecule.

And, incidentally, a virus is probably a chemical. It is not a living organism.

Mr. VOLKMER. All right.

Now, Doctor, I would like to ask you, who did you confirm your opinion with about the nature of the product as to whether or not it falls within the recombinant DNA guidelines, Baylor IBC?

Dr. Krr. Yes, sir, this is a common opinion for people working in the field. This is the way we view things. And specifically in a request for guidance from the IBC concerning certain aspects of the work which get a little bit more complicated than (the statement was reiterated by a member of the IBC at Baylor who is also in the department of virology, Dr. Janet Butell), that we considered these molecules to be viruses.

And, again, let me simply read section 3(d). Exempt for the guidelines are those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host—or a closely related strain of the same species—or when transferred to another host by well established physiological means, and also, those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, and so on.

I'm sorry, I read the wrong thing.

Mr. VOLKMER. I understand what you are reading, Doctor, and I understand what you are intending.

But my problem is that I have been informed that the Baylor IBC has now been reviewing this and does not necessarily agree with your interpretation. I haven't received a formal word yet, but I understand that there will be an announcement.

Do you have any comment on that?

Dr. Krr. I can only comment that there may be differences of opinion of people—there may not be unanimity. But this is the—the committee includes people from all branches of the college of medicine. This is the commonly held view among virologists working in this field.

Mr. VOLKMER. Now, as I understand also, as regard to this controversy, I have a letter [see appendix 3, p. 293] addressed to the NIH, Office of Recombinant DNA Activities, from Texas A&M University at College Station, where they don't agree also (this is from their IBC) with your interpretation. That's—we will furnish you with a copy of that for your review. And—

Dr. KIT. Mr. Chairman, I do want to emphasize that I regard the material as recombinant DNA derived, but I do not regard the virus as a recombinant DNA.

Mr. VOLKMER. Perhaps you may be stretching a point a little bit—maybe not, we will see.

Now, I understand also—well, first, do you know a Dr. Charles Livingston at the Texas Agricultural Experiment Station?

Dr. KIT. Sir, I have never met him—I know of him.

Mr. VOLKMER. As I understand, he was asked permission to do the experiment on the Maddox farm and he was told not to proceed—it was premature; that proper authorities hadn't been notified, and that all the safety data weren't in yet. Did Dr. McConnell of Texas A&M ever tell you the agricultural experiment station's refusal to participate in the Maddox field test?

Dr. KIT. No, sir, and to my knowledge, Dr. Livingston never participated in the Maddox field test.

Mr. VOLKMER. I know he didn't. But did Dr. McConnell ever tell you of that refusal?

Dr. KIT. No.

Mr. VOLKMER. At this time, I will go to other members. I have taken more than my time. I will recognize the gentleman from Iowa, Mr. Bedell.

Mr. BEDELL. Dr. Kit, since this contract also applies to Baylor, I presume it's public information. Would you tell us what the remuneration is for Baylor or yourself in regard to this contract? Was there a cash payment ahead, or what was it?

Dr. KIT. Would you repeat your question, sir?

Mr. BEDELL. I want to know what the contract is that you have in the licensing of your patent. The money involved.

Dr. KIT. I—I am the principal investigator of a contract from NovaGene to the Baylor College of Medicine. The contract is a contract for research—

Mr. BEDELL. No, no, you have a contract with Dr. Bartkoski, do you not?

Dr. KIT. Oh, oh, oh, yes.

Mr. BEDELL. Your firm. And what are the terms of that contract?

Dr. KIT. That's a contract between TechAmerica and NovaGene. I am not a party to it.

Mr. BEDELL. Well, what is Baylor—where does Baylor fit in? I thought they owned half the patent.

Dr. KIT. Baylor has—Baylor has a contract with the—with NovaGene.

Mr. BEDELL. OK. What is your contract with NovaGene?

Dr. KIT. My contract with NovaGene?

Mr. BEDELL. Baylor's contract with NovaGene.

Dr. KIT. Baylor has a contract with NovaGene that provides for NovaGene providing Baylor with research funds for the execution of the project related to vaccine development.

Mr. BEDELL. No, no, I'm talking about the patent. Baylor owns half the patent. Who's going to get the rewards from the patent and what are those rewards, is my question, Dr. Kit?

Dr. KIT. I see, I see. The rewards from the patent will—will be distributed between Baylor and NovaGene according to the contract. Baylor's portion—proportion of it, I believe, is 37½ percent.

Mr. BEDELL. No argument about the percent. I want--I want to know how many dollars are involved in this for you or your son, because this is a--to me is a--major part of the issue. Is this something that has large dollars involved, or is this something that's a small amount of money anyway?

It's supposed to be public--if Baylor gets--

Dr. KIT. I'm sorry, I don't understand.

Mr. BEDELL [continuing]. Half of it, the public must be able to know--

Dr. KIT. I don't understand your question. If--if there's a great deal of vaccines sold there will be royalties.

Mr. BEDELL. OK, what is the royalty agreement?

Dr. KIT. The royalty agreement is that--that for every dollar that might be earned in royalties, that Baylor will receive 37½ percent.

Mr. BEDELL. And what are the royalties percent of sales?

Dr. BARTKOSKI, can you tell us?

Dr. KIT. I really don't know, sir.

Dr. BARTKOSKI. Yes, the royalties split between TechAmerica and between NovaGene is 50-50.

Mr. BEDELL. Fifty-fifty between TechAmerica. You don't own the patent, do you?

Dr. BARTKOSKI. No, but you see--see, you are asking two different things. They have assigned us--

Mr. BEDELL. The patent?

Dr. BARTKOSKI. The--

Dr. KIT. No.

Dr. BARTKOSKI. No, the patent is not assigned to TechAmerica. TechAmerica is simply more or less licensed by NovaGene to develop and market the Omnivac vaccine.

Mr. BEDELL. That's right.

Dr. BARTKOSKI. OK. That is--that is what our contract is with NovaGene. We have nothing--we have no dubs, so to speak, on the patent. That belongs to NovaGene.

Mr. BEDELL. I am a businessman, I understand that.

Dr. BARTKOSKI. OK.

Mr. BEDELL. But you are going to pay them a certain amount of your sales as royalty?

Dr. BARTKOSKI. Right.

Mr. BEDELL. What is that percent?

Dr. BARTKOSKI. As I said, 50 percent.

Mr. BEDELL. Of your sales?

Dr. BARTKOSKI. Of our sales.

Mr. BEDELL. You mean if you sell a dollar's worth of product, they get 50 cents?

Dr. BARTKOSKI. After certain things are excluded out. I am not familiar with all the specifics, but, yes, it is.

Mr. BEDELL. About how much do you anticipate your sales being in this?

Dr. BARTKOSKI. You will have to speak to our marketing people. The sales figures have been fluctuating and I am not familiar with any of--of the exact figures. I can't--I couldn't give you an accurate figure.

Mr. BEDELL. Would it be in the millions of dollars?

Dr. KIT. I hope so. [Laughter.]

Dr. BARTKOSKI. Hope so.

Mr. BEDELL. I guess the point is, then, this is big bucks.

Dr. BARTKOSKI. Well——

Mr. BEDELL. If they are getting 50 percent—I never heard of such

a——

Dr. BARTKOSKI. Psuedorabies is a major disease.

Mr. BEDELL. Sure. And they are getting 50 percent.

Dr. BARTKOSKI. I mean——

Mr. BEDELL. OK. So that this is large dollars involved, then. Is this the sales we have? You are already selling the product?

Dr. BARTKOSKI. The vaccine has been licensed since January 1986.

Mr. BEDELL. What have your sales been from January to April?

Dr. BARTKOSKI. It would just be an approximate figure. So I really would be uncomfortable—I don't have an exact figure.

Mr. BEDELL. Apparently it has been reported in the papers, is that correct?

Dr. BARTKOSKI. There's been a lot of things reported in the paper. But what the paper has done is just taken general numbers. We had made the comment at one time that——

Mr. BEDELL. OK. No point in pursuing it any further.

I guess the issue here is that apparently this could be a very, very lucrative thing for the people that hold the patent rights, I would say. At least it's my impression that that's the case.

Dr. KIT. Of course.

Mr. BEDELL. OK.

Is it correct, Dr. Kit, that something genetically derived could be dangerous if released into our environment?

Dr. KIT. I think you are asking a hypothetical question. Any product that has a toxin—a diphtheria toxin, or other toxins, whether derived through genetic engineering or any other way, are dangerous.

Mr. BEDELL. OK.

Dr. KIT. Botulin toxin, for example, and so on.

Mr. BEDELL. So some of them could be dangerous——

Dr. KIT. Of course.

Mr. BEDELL [continuing]. And there's that concern expressed; is that not correct?

Dr. KIT. Very much so.

Mr. BEDELL. Sure.

And NIH did set out some guidelines as to what was to be done?

Dr. KIT. Yes.

Mr. BEDELL. Before you conducted those experiments at Texas A&M, did IBC give you approval that this did not fall within those guidelines? Did you get specific approval from that committee that this did not——

Dr. KIT. The experiments conducted by me at the Baylor College of Medicine were performed with registration with the IBC. I collaborated with many investigators around the world and it is very common to do so.

Mr. BEDELL. But that's not my question, Dr. Kit. I know you collaborate——

Dr. KIT. My answer is that I assume that the individuals carrying out the experiments do so in a proper manner in their home institution.

Mr. BEDELL. OK.

These were conducted at Texas A&M?

Dr. KIT. Yes.

Mr. BEDELL. Were—did you conduct the experiments?

Dr. KIT. No.

Mr. BEDELL. I thought you did. I thought you—

Dr. KIT. No, I did not. I just corrected that, sir.

Mr. BEDELL. Who—who conducted the experiments?

Dr. KIT. The experiments at Texas—in the Texas A&M University system, and it's a broad system involving several cities—were conducted by Bruce Lawhorn, Texas Extension Service veterinarian, and by Charles Livingston, and by Stewart McConnell.

Mr. BEDELL. By Stewart McConnell.

Dr. KIT. Yes.

Mr. BEDELL. Who—I don't understand. If it was your patent and your proposal, that you didn't have anything to do with conducting the experiments to test this—this material.

Dr. KIT. I supplied the virus. I didn't conduct the experiments.

Mr. BEDELL. Well, did somebody contract with Texas A&M to conduct them or did they just do it because they like to conduct experiments, or why did—

Was there no connection between you and the experiments that were conducted at Texas A&M?

Dr. KIT. Sir, there has been an ongoing collaboration between myself and investigators at Texas A&M, and other members of the Baylor College of Medicine and Texas A&M, since 1982. And I have submitted reprints of the work carried on on bovine herpesvirus type 1, which causes infectious bovine rhinotracheitis and—infectious vulvovaginitis. That was also conducted at Texas A&M.

Mr. BEDELL. Yeah, but—

Dr. KIT. There's nothing unusual about our conducting experiments.

Mr. BEDELL. But there's something that we need to understand as a committee here.

Dr. KIT. Right.

Mr. BEDELL. And that is, apparently there is concern over the possible release into the environment of some genetically derived products. And that's a very great concern in our society, that there were experiments conducted, as I understand it, at Texas A&M.

Dr. KIT. Yes.

Mr. BEDELL. On this virus that you developed and that you hold some of the patent rights and have a chance to get very, very wealthy over.

Dr. KIT. The patent rights belong to the Baylor College of Medicine—not to me.

Mr. BEDELL. Well, I thought half of the patent rights belonged to this NovaGene that you are part of.

Dr. KIT. No, I'm not part of it. I don't have any—any role in NovaGene other than that of scientific adviser. My older son is the president of NovaGene.

Mr. BEDELL. OK. Well, they own part of the patent rights, do they not?

Dr. KIT. Yes.

Mr. BEDELL. OK. So your son then—I don't care where it is. But there's that possibility.

Then there were experiments conducted, as I understand it, at Texas A&M.

Dr. KIT. And in other locations around the world in collaboration with me.

Mr. BEDELL. OK. I understand that there's great question as to whether those experiments that were conducted at Texas A&M were conducted properly under the guidelines. And you say that they were, in your opinion; and other people say that they think they were not.

Now, we are not here to judge that, and I am not enough of a—of a specialist to have any idea whether that's correct or not.

But the legitimate concern, I think, that we can legitimately have, is whether or not whoever was responsible for those experiments properly checked with NIH and the other authorities to get adequate clearance; or whether they did it simply because it was your opinion that they did not have to.

You have to admit that there is a good reason for you having an opinion that you shouldn't have to, because of the fact that at least some of your family had a chance to get quite wealthy over what was going to occur.

And the problem is not just you, Dr. Kit. The problem is that if these experiments can be dangerous, and if a person such as you can make up their mind that these do not fall under the guidelines and, therefore, we don't have to worry about it, and can go ahead and have the experiments conducted. And if, indeed, the person that conducted the experiments has left Texas A&M and now come with NovaGene—as I understand is the case—then it isn't the question of whether this is a dangerous thing you did; it is, are we setting an example where other people will do the same darn thing with something that would be much more dangerous? And, secondarily, if indeed you were wrong, and you didn't check with NIH, or somebody didn't, as they should have, and made proper checks—and then as a result of that you are able to get your family wealthy, then it seems to me—it seems to me that we had better look at what our penalties are to see if we have adequate penalties so that this system is going to work in a manner that adequately protects the public.

That's my opinion from where I sit. It appears quite clear to me that there's great question as to whether what was done was done properly. And I don't want to take so much of the committee's time, but it is impossible for me to believe that since you were the ones to benefit, and so on, that you really didn't have anything to do with those experiments that were conducted under questionable circumstances at Texas A&M.

That's the thing—and those—that's the issue. We've got another issue with the Department as to how they license people. But it seems to me that in this part here that we have a situation that's not too greatly different from some of the checks we are making in regard to White House people getting money, and so on. The exam-

ple we set—if what you can do is get rich by not following—not checking in the guidelines, and not be penalized for doing so, it seems to me we've got a heck of a problem on our hands.

Dr. KIT. Let me—let me comment to three levels.

Mr. BEDELL. Sure.

Dr. KIT. First, all scientific collaborations assume that the individual with whom you are collaborating—whether he is at the same university in a different department, or at another university—follows the rules of his own institution. That's the first thing.

And I assumed that all of my collaborators: Dr. Pirtle, Dr. McConnell, Dr. Lawhorn, and others, are following the rules of their university. Dr. Brad Thacker of Michigan State University who did the testing for TechAmerica during the licensing process was following the rules of the University.

The second thing—I think, again, you are losing sight in this hypothetical example of the fact that the starting material for our work is a licensed vaccine, is a vaccine that is already safe. We improved the safety.

Mr. BEDELL. No, that has absolutely nothing to do with it. The issue is—

Dr. KIT. Well, the first thing—

Mr. BEDELL. The issue is whether the guidelines were followed. And you say they were, and other people—

Dr. KIT. I say they were.

Mr. BEDELL [continuing]. Say they weren't.

Dr. KIT. And the third thing—

Mr. BEDELL. But that's—you know, I could say—I could say that it's perfectly all right for me to go shoot somebody. But they say no, you can't go shoot somebody. And even though I say it's all right, that doesn't make it all right. We have to go by—and we're not here, we don't know. But if the ruling should come that this was a violation, then the question is whether the penalty is going to be sufficient enough to deter other people from going ahead and doing something in order to get rich, or whether the penalty is so small that it still makes sense for somebody not to pay any attention to the rules and regulations. It seems to me that's the whole issue here.

But apparently you are testifying that you really didn't have anything to do with those tests that were made at Texas A&M on these hogs. Apparently, that was—

Dr. KIT. I am certainly testifying—

Mr. BEDELL [continuing]. Other people.

Dr. KIT. I am certainly testifying that I'm not familiar with the Texas A&M system, the mechanisms, by which they arrive at—at the approval of experiments to be conducted there.

Mr. BEDELL. And all you did was turn it over and asked them if they would check to see if it was all right?

Dr. KIT. No, I didn't turn over and ask them. I had an ongoing collaboration, which has existed since 1982—and still exists.

Mr. BEDELL. Well, the issue—

Dr. KIT. And the other thing is it seems to me that you are making us rich pretty quick. I don't think it is quite that easy to get rich.

Mr. BEDELL. Well, if you've got a 50-50 contract—

Dr. KIT. I don't have a 50-50.

Mr. BEDELL. No, but they're going to pay 50 percent of the revenues in—for the licensing of a patent. I've been a businessman. I never heard of anything even closely approaching that. That's a tremendous, tremendous patent royalty that they're paying. And if you are going to get 50 percent of their sales after some deductions, that's really an unbelievable contract, in my opinion.

Dr. KIT. Sir, may I—

Mr. BEDELL. And certainly, this is—you surely think this is a good product or you wouldn't have done all the work. So—

Dr. KIT. Sir, may I—

Mr. BEDELL [continuing]. But that's beside the point.

Dr. KIT [continued]. Respectfully point out that I'm also not a businessman. I've nothing to do with the business relationships. I did not negotiate any of the business relationships. I have not been a party to them.

Mr. BEDELL. I yield back the balance of my time.

Mr. VOLKMER. The Chair recognizes the gentleman from Wisconsin.

Mr. SENSENBRENNER. Thank you, Mr. Chairman.

First of all, let me say, I don't see anything wrong with getting rich by inventing something new and better than what has existed before.

I think the issue that we have to look into in this committee was whether the rules were violated—and if they were, how to prevent future violations from occurring in order to protect the public. So my line of questions is going to be along that line rather than how the royalties got split.

Dr. Kit, is Omnivac safer and more effective than similar vaccines made by traditional methods?

Dr. KIT. It is measurably safer by direct experimentation in sensitive hosts such as mice and rabbits, and in lambs. The reason that the lamb experiment was initiated is because of reports in the literature, both in the United States, with one of the companies that now markets a modified-live virus; and also reports in Europe of accidents with very low doses of vaccine which killed lambs. They were vaccine induced accidents.

So, that was really one of the reasons that—that our virus was tested in the lambs, to obtain information as to whether in fact it was safer. And it is measurably safer and also safer for other reasons.

Mr. SENSENBRENNER. Was this knowledge known before the testing or was it learned as a result of the testing?

Dr. KIT. The knowledge that it was safer was learned as a result of testing.

Mr. SENSENBRENNER. Well, from that testimony it seems to me that testing under controlled circumstances should always be required since one is never sure what the results would be for even a relatively safe organism. And I think that your testimony has proven that point.

Now let me ask you this: Are you familiar with the NIH/RAC Guidelines and the reasoning behind the establishment of those guidelines?

Dr. KIT. Yes, indeed, sir.

Mr. SENSENBRENNER. Do you believe that you violated those guidelines?

Dr. KIT. No, sir.

Mr. SENSENBRENNER. Why or why not?

Dr. KIT. I—I believe I did not violate the guidelines because all the experiments that I conducted were conducted with notification of the IBC, which agreed that the materials being used in the experiments were exempt. And in addition to that, that all of the other experiments that were conducted with my virus by collaborators were done according to the USA—USDA guidelines. And also, as I indicated before, because the method of construction of the virus doesn't really fit the NIH definitions of a recombinant virus.

May I, sir, just add one point—

Mr. SENSENBRENNER. Surely.

Mr. KIT [continuing]. To your previous question.

One of the other reasons for confidence in the thymidine kinase-negative pseudorabies virus vaccine was the roughly 6 years of experiments conducted by myself and by other individuals in the United States and in England on thymidine kinase-negative mutants of herpes simplex virus type 1, herpes simplex virus type 2, herpes tamarinus, and bovine herpes type 1, also known as infectious bovine rhinotracheitis virus. All of the preceding studies clearly showed that thymidine kinase-negative mutants that are not deletion mutants, but only are spontaneous or mutagen-induced mutants carrying point mutations, are safer in experimental systems—experimental mice, guinea pigs, and rabbits, than the corresponding laboratory viruses that do have a functional thymidine kinase gene.

Mr. SENSENBRENNER. Well, let's get back to the guidelines.

Section 3(a) of the Federal Register notice dated November 23, 1984, identifies experiments which require specific RAC review and NIH and IBC approval before initiation of the experiment. Included in these experiments and defined in section 3(a)(II) are those experiments which would—which result in deliberate release into the environment of any organism containing recombinant DNA except certain plants described in appendix L.

You are talking as a scientist—I am talking as a lawyer now.

Dr. KIT. Certainly.

Mr. SENSENBRENNER. Do you believe that the field testing of all products made using recombinant DNA techniques should fit under these guidelines?

Dr. KIT. Yes.

Mr. SENSENBRENNER. OK.

Now, you said that you notified the IBC that you were going to do this experiment. Did you ever receive an approval from the IBC?

Dr. KIT. Yes, that I would do the experiments in my laboratory. That's what was—that's what the notification was.

Mr. SENSENBRENNER. OK.

Dr. KIT. But—but, sir, again, I don't know of any virologists who regard vaccination on—especially under conditions where there's no shedding—as release into the environment, and especially if the vaccination occurs under carefully controlled conditions.

The progeny viruses in the urine consist of a population of poliovirus particles in which one in a hundred thousand is a revertant to virulence. That is, 1/100,000 of the poliovirus particles in the urine can cause disease and no longer has the properties of the vaccine virus on the sugar pill. In the United States there are very few cases of polio. The cases that do arise are vaccine-induced in either the individuals who have taken the sugar pill—or their contacts. Is that released into the environment?

Mr. SENSENBRENNER. Well, you know, again, one of the problems of a committee that does not contain any scientists reviewing scientific policy is that we can't answer a question like that. We have to rely on experts.

I think what we are looking into is procedures here. And I just want to get some facts relative to the procedures and whether they were complied with.

Now, if you notified the Biohazard Safety Committee prior to the laboratory test, why didn't you notify the committee when you field tested the vaccine on the Maddox farm of June of 1984?

Dr. KIT. I did not field test the virus. The virus was field tested on a quarantined farm containing pseudorabies by Texas Extension veterinarians after consultation with about 10 other veterinarians and under the guidance and approval of the Texas Animal Health Commission.

Mr. SENSENBRENNER. Yeah, but—you know, I see some finger pointing around. You say you didn't do it, somebody else did—

Dr. KIT. No.

Mr. SENSENBRENNER [continuing]. And, you know, I am just trying to find out who is responsible for the approval to go ahead with the field test for the vaccine.

Who was responsible for that?

Dr. KIT. John Holcombe, the executive director of the Texas Animal Health Commission.

Mr. SENSENBRENNER. Did you know about the fact that a field test would take place?

Dr. KIT. Yes.

Mr. SENSENBRENNER. Did you give them a vaccine to go ahead and field test it?

Dr. KIT. Yes.

Mr. SENSENBRENNER. And yet you didn't notify the committee prior to the time the field test took place.

Dr. KIT. No, sir. And in addition, we have the guidance of the Veterinary Services Memorandum No. 800.68, which also clearly says that, "In normal husbandry and laboratory practices, veterinary biological products are not considered to be released into the environment."

Mr. SENSENBRENNER. Uh-huh.

Dr. KIT. Now, again, I am not a lawyer—I am a scientist, and it isn't up to me to determine.

Mr. SENSENBRENNER. I am looking through your voluminous testimony—and page number D-2, second paragraph, it said: We submitted an experimental vaccination/eradication plan drafted by Dr. Stewart McConnell and Bruce Lawhorn.

It means that you submitted that vaccination plan, and yet you are saying you don't know anything about this vaccination that occurred on the Maddox farm.

Dr. KIT. I didn't say I don't know anything about it. I participated in the plan by providing the virus and discussing the safety of the plan before it was approved.

What section is that?

Mr. SENSENBRENNER. It's page D-2.

Well, you brought up USDA Veterinary Services Memorandum No. 800.68, which states that in normal husbandry and laboratory practices, the veterinary biological products are not to be released into the environment. The memorandum is dated December 4, 1984, and yet, the Maddox farm field test occurred 6 months earlier in June of 1984.

Given this, wouldn't the field test have been classified as an environmental release subject to the NIH/RAC approval under the guidelines in effect at that time?

Dr. KIT. I don't think so. I think that the—that the Veterinary Services Guidelines reflect the thinking of veterinarians, that vaccination under these conditions is not release into the environment. It affirms it.

Mr. SENSENBRENNER. Well, do you or don't you consider the experimental vaccination of 1,300 swine an environmental release?

Dr. KIT. No, I do not.

Mr. SENSENBRENNER. Well—

Dr. KIT. Sir—

Mr. SENSENBRENNER [continuing]. I think that's contradictory, sir, with all due respect.

Dr. KIT. No, sir. If you read the plan, you will see that on this quarantined farm, very careful precautions were taken with regard to where the animals were, with regard to what would happen to them, and how they were to be disposed of, and so on.

And, again, all of the experiments were done with the knowledge of the experiments already completed at the National Animal Disease Center of the Department of Agriculture, which indicated that there is no shedding.

Mr. SENSENBRENNER. Well—

Dr. KIT. How can you release—how can you release into the environment a vaccine which is manufactured and sold similar to one manufactured and sold—except better and safer—on a farm which has disease in an environment where there is disease?

Mr. SENSENBRENNER. Well, Dr. Kit, you know, we are talking about genetic engineering. And I think you can consider yourself a very lucky man, because if this test had not been a success, there would have been some very serious environmental consequences and agricultural consequences. Because it was a success, there were not those consequences.

But, again, when we are dealing with something as imprecise and as experimental as genetic engineering, you don't know what is going to happen. And, again, because it did work, you were pretty lucky, and I—

Mr. BEDELL. Will the gentleman yield?

Mr. SENSENBRENNER. I yield to the gentleman from Iowa.

Dr. KIT. Sixteen percent of the wild pigs in east Texas—18 percent—in the last test are infected with pseudorabies. Sixteen percent of the wild pigs. That's the environment.

Mr. BEDELL. If the gentleman will yield?

Mr. SENSENBRENNER. I yield to the gentleman from Iowa.

Mr. BEDELL. First of all, I commend the gentleman for his questioning. I think it has brought out some very important facts.

Mr.—Dr. Kit, did you get approval from Baylor to ship the vaccine for these experiments that were conducted at Texas A&M?

Dr. KIT. No, sir.

Mr. BEDELL. Do you feel you should have, or should not have?

Dr. KIT. No, sir.

Mr. BEDELL. Did they own half the patent?

Dr. KIT. All scientists purchase recombinant DNA plasmids from suppliers, such as New England Biolabs, and they exchange recombinant plasmids with other scientists without the involvement of IBC's.

Mr. BEDELL. That's not the issue.

Dr. KIT. We send viruses and cells to other investigators. It does not require administrative approval.

Mr. BEDELL. But they had half interest in this, did they not?

Dr. KIT. Uh—

Mr. BEDELL. Well, of course they did.

Dr. KIT. I don't—I don't understand the—

Mr. BEDELL. They had half interest in the patent.

Dr. KIT. So?

Mr. BEDELL. And—and, so you—

Dr. KIT. That has nothing to do with my procedures of sending cells and viruses to other investigators, or receiving them.

Mr. BEDELL. Well, I think there's some question as to whether they will be liable or not in case what was done was wrong.

Mr. SENSENBRENNER. May I reclaim my time?

Dr. Kit, are you under investigation by either Baylor or Texas A&M as a result of this transaction?

Dr. KIT. Not to my knowledge. I have—I have answered questions to the Baylor Institutional Biosafety Committee.

Mr. VOLKMER. Will the gentleman yield on that point?

Dr. KIT. I don't think I am under investigation, no.

Mr. SENSENBRENNER. I yield to the gentleman from Missouri.

Mr. VOLKMER. Well, it's my understanding that there may be a statement issued from Baylor College of Medicine today—a statement of Baylor College of Medicine today to the effect that the Baylor College of Medicine IBC disagrees with Dr. Kit as to the implications of the release on the—what was it—Maddox herd, as to whether or not that that should have been subject to approval by the IBC at the Baylor College of Medicine.

Now, I understand that release is coming today.

Mr. SENSENBRENNER. One moment, Mr. Chairman.

I yield back the balance of my time.

Mr. VOLKMER. The Chair will recognize the gentleman from the State of Washington.

Mr. MORRISON. Thank you, Mr. Chairman.

We seem to have had a series of questions almost highlighted by the professions from which they came. As a farmer, I want to let

you gentlemen know that I am delighted the more money you make, the better off I am going to be—and I compliment you for your contributions to science and its improvement.

The key, of course, from this hearing, as far as I am concerned, is, what procedures are in place? Are they adequate? Were they—I guess I am a little less concerned at this point as to whether you followed what was precisely in front of you. At the moment, I am more concerned about what happens in the future.

I would like to ask a few questions of Dr. Bartkoski, and that is, with the procedures that you had in front of you as you worked with this material, were they relatively clear?

Dr. BARTKOSKI. Yes. When we begin licensure, we follow very precise regulations laid out by the Virus-Serum-Toxin Act in the publication which you have probably seen, called "The Code of Federal Regulations," Volume 9. And that lays out and defines those procedures which we must follow for the licensure for all of our better made biological products.

Mr. MORRISON. Was there any question at all in your mind as to which agency within USDA would review your license application?

Dr. BARTKOSKI. None whatsoever. I mean, we only—I mean, there is just one agency which reviews our license—the one that did grant it in the first place for—and that happens to be USDA/APHIS, the Animal and Plant Health Inspection Service.

Mr. MORRISON. Did—as you were moving through this process, did you—did you sense some change in the regulations? Were the regulations changing, or did they stay pretty firmly in place while you were going through the license procedure?

Dr. BARTKOSKI. They were pretty much in place all the way through.

Mr. MORRISON. How about the—when did you become familiar with the NIH/RAC Guidelines?

Dr. BARTKOSKI. We were familiar with those guidelines from the beginning.

Mr. MORRISON. Did you have any questions in your mind on conflict between those guidelines and USDA's compliance with them?

Dr. BARTKOSKI. No.

Mr. MORRISON. No question at all. OK.

Did—of course, you used the results of studies conducted by Dr. Kit as background information—

Dr. BARTKOSKI. Yes, we did.

Mr. MORRISON [continuing]. In the licensing process?

Did you ever conduct any independent tests, or any tests which might have varied with the work that Dr. Kit was doing?

Dr. BARTKOSKI. Yes, we conducted very significant tests. The background information that we receive—whether it is with this product or with other products—serves as just that background. We are still required to do testing as defined by those regulations which we have to follow. None of our testing was at variance with any of Dr. Kit's. But we had to perform tests beyond those. None of those tests, in fact, can be submitted—I mean, except for background data.

Mr. MORRISON. Did you have any idea at all at the time why USDA reclassified the vaccine as a recombinant organism?

Dr. BARTKOSKI. When that—when that reclassification took place, we had discussions with them. And the response is based on the memo—800.68, which we have just talked about—which says that regardless—and it is a memorandum for the USDA/APHIS branch, and it classifies anything using new technology as being recombinant. That was the response.

Mr. MORRISON. Is that an accurate classification in this case?

Dr. BARTKOSKI. Well, it is a classification for their housekeeping, for them to track licenses or those projects which they are, you know, happen to be working on at that point in time. For instance, a synthetic peptide—which is totally synthesized, which has nothing to do—you know, people make a piece. That would be a recombinant. I mean, they use that as a housekeeping mechanism. Certainly, you know, that is of their own choosing.

Mr. MORRISON. Did you have any concerns about the field testing which you have just described once this reclassification came about?

Dr. BARTKOSKI. No.

Mr. MORRISON. Did you have any concern at all about the validity of Dr. Kit's field test results since neither IBC or NIH approval had been granted for those tests?

Dr. BARTKOSKI. No, because prior to starting any of our own field tests, we did testing in confined quarters where we demonstrated the safety of the vaccine; where we demonstrated that it would work as a vaccine, that you could actually vaccinate an animal with it; and that then you could subsequently challenge that animal and that the animal would be protected.

We had also done back passage studies, which are very critical studies, because what those studies tell us is that if we do vaccinate an animal—and these are classical experiments that are done for all modified-live back—for all modified—live products. And that is, if you vaccinate an animal, and if you go back in and attempt to isolate the organism, and then continuously pass it back through—what happens? It's a very important question, because that is the mechanism by which one would envision reversion happening. And we had all those experiments done prior to starting any field tests.

Mr. MORRISON. All of this work reflecting your many years of experience—

Dr. BARTKOSKI. Yes.

Mr. MORRISON [continuing]. In this whole—this whole field.

Well, I thank you for your answers.

And, Mr. Chairman, thank you for the time.

Mr. VOLKMER. The Chair recognizes the gentleman from Iowa, Mr. Evans.

Mr. EVANS of Iowa. Well, thank you, Mr. Chairman. I think the matter has been very well covered by the testimony. I just have one short question.

I wonder if either of the witnesses at the desk can inform the committee of what the approximate losses per year are that are suffered by the pork industry due to pseudorabies.

Dr. BARTKOSKI. We can address that. The estimation is that approximately 10 percent of the animals have pseudorabies that are out there. And that amounts to 10 percent of approximately 60 to

70 million animals. The annual losses are in the neighborhood of approximately \$60 million per year to the pork industry.

Mr. EVANS of Iowa. Thank you. I yield back the balance of my time.

Mr. VOLKMER. I have a few additional questions, and then we will go to our next panel. But before I begin, I just want to see if, Dr. Kit, you agree with the general statement I am about to make, that prior to December 1984, and January 1985—in December, it is my understanding that USDA announced that it's time that they would follow, and in January 1985 did publish in the Federal Register, guidelines to be followed, and procedures where you have a product from recombinant DNA derived. And that prior to that time, USDA had informed publicly that applicants were to use NIH/RAC guidelines. Is that correct? Do you understand that?

Dr. KIT. I heard you correctly, I believe so, sir.

Mr. VOLKMER. And prior to that time, they were to use NIH—prior to them adopting their own.

Dr. KIT. I think they still use NIH guidelines.

Mr. VOLKMER. Right.

Dr. KIT. I don't think—I don't think the USDA, to my knowledge, has ever contradicted the NIH guidelines.

Mr. VOLKMER. All right.

Perhaps there appears to be some disagreement as to, I guess some basics. And those are going to have to be somewhere along the line, I would say, corrected, as we get more in the field of biotechnology—and that is, No. 1, what is actually a recombinant DNA derived product and needs to follow the guidelines. I think we are going to have to clarify that; No. 2, and I think the question of whether an inoculation to an animal is a release into the environment is going to have to be straightened and corrected because I think there's going to be areas of disagreement in both of those. And to show where—perhaps I, am, at least personally coming from, that we need to do this if we are going to have to be able to continue with the type of regulation that we presently have. Or else if this is not being able to be worked out, I am afraid you may end up with a lot more tighter regulation where this industry might be told to stifle—which some of us don't want to see, because we see end products that would be very beneficial, including possibly the end product here, as has been pointed out by the farmer—farm state representatives—

Dr. KIT. That's true.

Mr. VOLKMER. And myself, I have a lot of pork producers in my district. And I would like nothing better than to get rid of pseudorabies.

Dr. KIT. Yes.

Mr. VOLKMER. But, Dr. Kit, you want to say something now.

Dr. KIT. I just want to say the third area that needs classification is what is the meaning of exempt?

Mr. VOLKMER. Right, I agree.

Mr. BEDELL. Mr. Chairman?

Dr. KIT. And also, I don't remember who said that I was a very lucky young man. I imagine Columbus was pretty lucky that the world was round.

Mr. BEDELL. Mr. Chairman?

Mr. VOLKMER. The gentleman from Iowa, Mr. Bedell.

Mr. BEDELL. Dr. Kit, you said that there was no environmental release because there was no shedding. Is that correct?

Dr. KIT. That's one reason. There's no environmental release because there's vaccination. In—in—in a quarantined herd under quarantined conditions.

Mr. BEDELL. Wasn't—

Dr. KIT. What is the meaning of quarantine?

Mr. BEDELL. Wasn't—wasn't part of the test to determine whether or not there was shedding?

Dr. KIT. No, sir. The—well, this was—this was a—an additional piece of information that could be obtained.

Mr. BEDELL. But that—

Dr. KIT. But the experiments—

Mr. BEDELL. But that was part of the purpose of the experiment, was it not?

Dr. KIT. It was already demonstrated by the experiments carried out by Gene Pirtle at Ames, IA.

Mr. BEDELL. My question is—

Dr. KIT. That's what a shedding experiment is.

Mr. BEDELL. My question is: Was part of the test to determine whether or not there was shedding. Either yes or no, it was or it was not. Was part of it to determine it?

Dr. KIT. Yes.

Mr. BEDELL. So if part of it was to determine it, then there was—you didn't know it for sure before you had the experiment or there's no point in having done the experiment.

Dr. KIT. As a scientist, I don't know anything for sure.

Mr. BEDELL. Well, OK.

Then let me read also here: In 1985, TechAmerica wrote letters to the States of Illinois, Michigan, requesting permission to test its vaccine in those States. The letter stated that the tests would be intended to measure the safety of gene-deleted vaccine and that the vaccine virus—would be minimally excreted.

Yield back.

Dr. KIT. Yes. And it's important to note that I'm not the only investigator who has worked on pseudorabies. There are other laboratories who have also studied this with thymidine kinase negative mutants—not deletion mutants like my mutant was, but nevertheless thymidine kinase-negative mutants. And their results were exactly the same no shedding.

Mr. BEDELL. I want one—

Dr. KIT. Their data was also available to us.

Mr. BEDELL. I want one thing clear, and that is, pseudorabies is a serious problem. I think we owe a debt of gratitude to anybody that properly helps to solve that problem. The concern I have is where we may be headed if we don't have adequate controls on what's done.

Mr. VOLKMER. All right.

Dr. Kit, a couple more questions.

Dr. KIT. OK.

Mr. VOLKMER. You state in your testimony that NovaGene is apparently preparing another form of the vaccine that has additional gene deletion.

Has this version of the vaccine been tested outside the laboratory?

Dr. KIT. Yes. For one thing, the vaccine has been turned over to TechAmerica and has been tested under TechAmerica facilities in mice, and I think in pigs.

Dr. BARTKOSKI. Do you want me to respond?

Mr. VOLKMER. I ask Dr. Bartkoski, then, the same thing.

Dr. BARTKOSKI. We have not done any field studies. We have just—we have worked with it only within the laboratory to this date. What we have done, is we have—

Mr. VOLKMER. Before you did even the lab tests, did you seek any approval for the continuation of this testing on this?

Dr. BARTKOSKI. From—from who?

Mr. VOLKMER. From either USDA or NIH.

Dr. BARTKOSKI. Not from the NIH. With the USDA, whenever you work with an animal virus, normally the traditional mechanism would be that you let them know that you would be bringing that particular class of agent into your facility, because we are licensed. We had had prior approval for a number of years to bring the agent within and to keep it—and we are allowed to do that as long as it stays only within a research portion of the licensed facility. That is the only area where it has been.

The NIH Guidelines, we did review it by our own BioSafety Committee and that is as far as we have gone.

Mr. VOLKMER. But your BioSafety Committee has reviewed it so far?

Dr. BARTKOSKI. Yes.

Mr. VOLKMER. Prior to doing field testing, do you feel that there will have to be an application made to USDA?

Dr. BARTKOSKI. Yes. What we will do—and we absolutely have to make an application before we will do any field testing. Any testing that we do besides routine laboratory manipulations of an organism, we seek—we notify the USDA, let them know that we are going to pursue licensure of that particular organism, or that particular disease more properly. And following that, all protocols that are going to be utilized will be sent to them prior to the conduction of those tests; and they will be approved before we ever start.

Mr. VOLKMER. What you—who you plan to go through, of course, is APHIS?

Dr. BARTKOSKI. Yes. We are required to do that by the Virus-Serum-Toxin Act to go through APHIS. That is the only, I guess, legally authorized agency to license us in a veterinary biological that I am aware of.

Mr. VOLKMER. All right. Then we will have to take it up with—we will have to take it up with USDA as to whether or not the APHIS or USDA notifies ARRC and ARRC has any review of the matter. That's another question. That doesn't involve you, I understand that.

Dr. KIT, on the proposal to test a vaccine in Florida—to use it in Florida because of Florida panther—has the IBC been consulted at Baylor or University of Florida to decide on a proposed test?

Dr. KIT. The letter states that this is a preliminary proposal to me. And, yes, the administration of the Baylor College of Medicine, the IBC, and just about everyone else, has been consulted. There is

no plan at the present time that is fully drawn up. There will be, and at that time, just as seven veterinarians tested the vaccine that I designed—redesigned, I guess—the more people that test it and find it satisfactory, the better, from my point of view. The more people that review it, fine. I mean, I'm delighted to have NIH/RAC or—the Department of Agriculture Recombinant DNA Committee, and all other interested parties, review it.

Mr. VOLKMER. All right, one last one, for you, Dr. Kit.

Did anyone, during the 1983-84 timeframe—any of your colleagues ever comment directly to you that you may not be in compliance with NIH/RAC Guidelines?

Dr. KIT. No, sir, quite the contrary. One thing that has not been brought out here is that there were press releases from the Baylor College of Medicine concerning my work from 1982 to the present time. And I believe some of those press releases have been brought here.

Second, I made presentations of the work, and Bruce Lawhorn made presentations of the work. He made it on three separation occasions to pork producers. I made presentations within 2 months of the start of that experiment to an International Herpesvirus Congress attended by about 1,000 investigators.

In November, I was invited by the chairman of the department of virology at the Baylor College of Medicine to present the work at the symposium attended by the USDA, industry, and other scientists.

In April of 1985, I went to Morocco for a Congress dealing with the impact of infectious diseases on the developing countries of the world. And I presented the work there, too.

I find it very strange that so many years later, when this, to me, is even water under the dam, that the questions are raised. I can't help wondering whether there's concern of some of the people who did not come to a business agreement with NovaGene on the vaccine—that perhaps they will lose a little bit of the market. And that some of the recriminations that are involved may be related to that.

Mr. VOLKMER. Well, thank you very much, Dr. Kit.

Dr. BARTKOSKI, at the time that USDA reviewed TechAmerica's application, were there any conversations between TechAmerica and officials of the Veterinary Service Division regarding whether the vaccine should be classified as a recombinant organism?

Dr. BARTKOSKI. Not early on. Not when we first submitted our information in the end of December 1984.

Mr. VOLKMER. Well, did you originally at any time consider it a recombinant DNA derived product?

Dr. BARTKOSKI. Recombinant DNA derived using recombinant DNA technology, yes. A recombinant DNA molecule; no.

Mr. VOLKMER. Were they—was USDA notified as to what you felt the differences were?

Dr. BARTKOSKI. We did not spell out the differences. What we did do is when we submitted the preliminary license application, we also sent to them at that time publications from Dr. Kit which outlined in some detail how the organism was—was generated. And they had all that information from, really, the first submission that we had sent to them.

Mr. VOLKMER. Now, when the USDA did classify it as a recombinant organism, did they do it on a basis of any new information that you had sent to them?

Dr. BARTKOSKI. Not that I am aware of.

Dr. KIT. It is not classified as a recombinant organism. It's classified, I believe, as a recombinant-derived organism.

Mr. VOLKMER. Recombinant-derived organism.

Dr. KIT. Yeah.

Mr. VOLKMER. And USDA, however, as I understand it, has we will find out from Dr. Bentley—but it is my understanding, has classified it as a recombinant organism.

Dr. BARTKOSKI. They put an R designation on our product code. We've got a product code for everything, and the R designation defines it as recombinant, and that's all that it does say.

Mr. VOLKMER. All right.

Under the USDA's own guidelines, once a product is classified as recombinant. Is TechAmerica aware that USDA's Agricultural Recombinant DNA Research Committee was charged with reviewing proposals, to test and release these organisms?

Dr. BARTKOSKI. No.

Mr. VOLKMER. You weren't aware of that?

Dr. BARTKOSKI. I am not aware of their internal procedures?

Mr. VOLKMER. Pardon?

Dr. BARTKOSKI. I am not aware of their internal procedures; no. We deal strictly with APHIS branch.

Mr. VOLKMER. Well, you are not aware, then, of their guidelines—USDA's total guidelines for recombinant DNA product licensing review and testing?

Dr. BARTKOSKI. Which guidelines? Are you referring to the proposed guidelines that were published in, I believe—

Mr. VOLKMER. January of—

Dr. BARTKOSKI. Of 1984.

Mr. VOLKMER. 1985?

Dr. BARTKOSKI. Or 1985.

Mr. VOLKMER. December 1984 or January 1985, in that time frame?

Dr. BARTKOSKI. We certainly received copies of those. But those are just optional guidelines, and our guidelines really come directly from the APHIS branch.

Mr. VOLKMER. Well, so we know in the future, it is my understanding—and perhaps you can check with someone else, either your own counsel or USDA—but it is my understanding, according to USDA guidelines, that the ARRC, as we call it, was supposed to be notified and reviewed, for the purpose of review, any testing and releasing of such organisms.

Dr. BARTKOSKI. Yes, that is true. But that notification is not done by TechAmerica to ARCC. It is done internally, I believe, by APHIS to ARRC.

Mr. VOLKMER. You are taking the position that it is up to USDA to take care of their own house operations, basically?

Dr. BARTKOSKI. I mean, I—I mean, I cannot get involved in USDA's internal—it is a very large Department. We have a difficult enough time with the APHIS branch.

Mr. VOLKMER. All right, OK. [Laughter.]

OK, right.

Now, at the time that TechAmerica was negotiating with—

Dr. BARTKOSKI. NovaGene?

Mr. VOLKMER. NovaGene, did you consider the results of the experiment on the Maddox farm as part of your determination whether or not to go ahead with the agreement to market the product?

Dr. BARTKOSKI. We really looked at the whole package. But the actual testing that was started at the Maddox farm started later on in the process, and really, the data was—there was little data at that point in time, and there was not a significant amount for us to base the decision on. The actual decisions that were probably used in signing the agreement were just the data generated prior to the Maddox farm study. I mean, we were familiar with pseudorabies virus as a disease. We were familiar with the need for a new and a better vaccine. We were familiar with the disease as we now know it within the environment.

We were familiar with Herpesviruses as a group—some of the characteristics of them.

We were familiar with the thymidine kinase work.

And it was this body of data, really, and all the stuff prior to the Maddox farm that had the major impact on whether or not we would pursue this contract with NovaGene.

Mr. VOLKMER. Do you agree or disagree with the USDA classification that the—

Dr. BARTKOSKI. You mean—

Mr. VOLKMER [continuing]. The matter is—I'd say not derived, but is a—

Dr. BARTKOSKI. Calling our product recombinant?

Mr. VOLKMER. Now, recombinant organism, let's put it that way.

Dr. BARTKOSKI. Yes and no. It is going to have to be a two-part answer. Yes, understanding their mechanism for tracking new biotechnology products as they—and if you look at that memorandum, that is what they call them. They—you know, products resulted from new biotechnology. Yes, I do understand it from that.

As a definition, to call our product recombinant, it would not be considered, I don't think, by anybody's regulation or anybody's guidelines, as a true recombinant organism. And we get into this problem with terminology again, as you said; we may better need to define exactly what we were saying.

Yes, I agree, and I certainly understand their need for tracking "new biotechnology products," because they are, you know, for them, represent maybe a different way of looking at licensing.

On the other hand, I do not necessarily agree that it is a recombinant organism because by all the definitions that I am aware of, it would not classify as a recombinant organism.

Mr. VOLKMER. The Chair recognizes the gentleman from California.

Mr. PACKARD. Thank you, Mr. Chairman.

I apologize for not being able to be for this most—the witnesses at this particular time—other commitments. But let me just ask one question that, hopefully, is not duplicative of other questions that have been asked.

Dr. Kit, perhaps, I would like you to respond.

Guidelines and regulations should be formulated to protect the public, of course. But, also, should be formulated in a way to facilitate the research, the production, and—and, ultimately, the marketing of products safely and appropriately.

In this situation, obviously, there has been some serious misunderstandings and misreading of regulations and—and guidelines.

What changes do you think should be made that would better accommodate good research and development in the—in the, particularly in the biomedical area? What changes would you suggest be made in guidelines or in educating researchers and institutions on the guidelines so that these kinds of problems that we find caught up here will not be repeated?

Dr. KIR. I think that the three areas that the chairman mentioned: better—better attention to definition would be very, very helpful to guide all of us; especially the area that deals with what is exempt, what is the meaning of exempt, and also the definition of what is release. I am a little bit of a quandary as to these areas.

It would help also if—if the guidelines that were formulated applied to products, whether developed by the latest state of the art, as I call it, technology, or more conventional technology. It seems to me that the ultimate aim of safety, of insuring the maximum safety on the continuum of "minumum risk" on the left all the way to "high risk" on the right, which is what we are after in the whole design of this, applies to all biotechnology products. Whether they happen to use the particular experimental techniques that I am using, or experimental techniques that others have used, or techniques that may emerge in the future.

Mr. PACKARD. Dr. Bartkoski, do you have anything to add to that?

Dr. BARTKOSKI. No.

The only comment that I might make is, you know, when we look at these regulations, as Dr. Kit said, we need to have a good interpretation of terminology. I think, as we saw today, for myself, we have a meaning which is generally considered maybe for scientific terms. We have a meaning that the chairman might give to recombinant DNA technology. And while they may be very much the same, they are certainly open to discussion. And I think for all of us, and if we are going to have these regulations, we need strong definitions and strong—or, good guidelines for following these. And we have to get away from utilizing portions of guidelines from different sources—putting them together to make a new set of guidelines for the interpretation of how experiments are to be conducted. I think we need a unified set of guidelines if that is how we choose to regulate biotechnology. What we tend to do is to pool together terms and parts and pieces from varying departments and regulatory guidelines, and then to make a new one to fit a particular interest—product. And I don't think that long-term is going to do any good for us to proceed in that manner.

It is going to be very difficult for a company like ourself to even know how to interpret whether or not to attack a new problem, for instance.

Mr. PACKARD. In the case of Omnivac, are you, either of you, aware of any intentional or negligent disregard of—of guidelines or of—of regulation?

Dr. BARTKOSKI. Speaking for TechAmerica, none whatsoever. We have followed every regulation.

Mr. PACKARD. Thank you, Mr. Chairman.

Dr. KIT. No, sir.

Mr. PACKARD. Thank you.

Mr. VOLKMER. The gentleman from Minnesota have any questions?

Mr. PENNY. No, thank you, Mr. Chairman.

Mr. VOLKMER. OK. I have one more question, Dr. Bartkoski.

In November 1985, after USDA had redefined the product—

Dr. BARTKOSKI. Right.

Mr. VOLKMER [continuing]. And called it a recombinant DNA organism, they asked you to notify the States where the vaccine had been tested that a vaccine had been classified as a recombinant organism. And although the vaccine was licensed in January 1986, documents provided to this subcommittee indicate that the States were not notified of the classification of vaccine until February of 1986.

Now, why was this delay, and were any tests carried out in the States between November 1985 and February 1986 before the States were notified the vaccine was classified as a recombinant organism?

Dr. BARTKOSKI. Field tests were ongoing at that point in time when the notification was made. No, I did not specifically write a letter to the States telling them that this was a recombinant that it had been given a recombinant designation, prior to that, before the initiation of any of the field studies.

We had sent them documentation and we had notified them that the virus was a product of genetic engineering—and we had notified them. We had submitted to them the publications and the work that was previously done. Following that notification from USDA, we did not send a letter out immediately.

But—you know, one thing must be understood, you know, we have contacted them—been in periodic contact with them orally. And that is—that was a constant mechanism.

Mr. VOLKMER. But you really did inform them that it was a product derived through genetic engineering?

Dr. BARTKOSKI. Yes, we did. And, in fact, we even sent them the publication so they can see that. And in our first field test, the first one in the State of Illinois, we had a meeting with all the State represent—State representatives, excuse me, that are concerned with pseudorabies control, and we outlined in some detail the tests and the nature of the virus prior to ever starting that.

Mr. VOLKMER. Did you use as a basis—advise them on a test, the Maddox test—when they did the field test for you all?

Dr. BARTKOSKI. Did we use that? What—the data that we had present to them, they had prior to that. They had copies of those publications—the one arising from the Maddox farm, yes. But the data that we really utilized was testing that had been conducted at Michigan State University was demonstrating, you know, that the virus—excuse me, that the vaccine did work to protect against pseudorabies. And we had additional data there on the safety that we had conducted internally. That data was really the prime data that was utilized for us to start our field testing.

We would not have been allowed to start it based on the Maddox studies.

Mr. VOLKMER. Following notification in February to the States where the States were notified that it had been classified as a recombinant organism, has that changed any of the attitude toward testing in those States?

Dr. BARTKOSKI. None whatsoever. In fact, most of them have had no problems and we are really pleased, I think, that new technology was finally reaching the marketplace. None had any comments.

Mr. VOLKMER. OK.

Would you know, or would you not know whether the Michigan State IBC was notified prior to the field test?

Dr. BARTKOSKI. Michigan State?

Mr. VOLKMER. Yes.

Dr. BARTKOSKI. Oh, you mean Michigan State University?

Mr. VOLKMER. Right.

Dr. BARTKOSKI. I can only, you know, tell you—I have spoken to Dr. Thacker, and he initiates his studies up through—he starts with the Animal Welfare Committee—that is automatically, then goes to the IBC. And as far as I am aware, yes, it did go through all the channels at Michigan State University. They were all aware of it.

Mr. VOLKMER. OK. Thank you very much.

I want to again thank both of you. It has been a full morning, and I appreciate your being here and testifying. I hope that we can clear these matters up as we proceed so that we don't necessarily, through Government action, and adverse, possibly, regulation, completely stifle the industry. But—and I recognize the potential to both pork producers and ultimately to consumers, both in health areas and in economics, the desirability of a pseudorabies vaccine that will eradicate, hopefully, pseudorabies from the swine population of this country and the whole world, would be—would be desirable.

But on the other hand, I think that you have to recognize from the attitude, I think, for the members—both the Agricultural Subcommittee that are here, and the Science and Tech Subcommittee—and, by the way, I am a member of both—that we want to make sure that as we progress down this road for biotechnology development of recombinant DNA that we do have assurances to the public that health and safety are protected. And that we make sure that people who are in this area do follow the guidelines—and if we need to, make corrections to those guidelines—as we go along we will learn along with everybody else and make—hopefully, make those corrections. But we—you understand, I think, Dr. Kit, now, why we are concerned. Do you not?

Dr. KIT. Yes, sir.

Mr. VOLKMER. All right.

Dr. Bartkoski—thank you very much, both of you. We will now proceed to our second panel.

Dr. BARTKOSKI. Thank you.

Mr. VOLKMER. Our second panel is—

Dr. KIT. Thank you, sir.

Mr. VOLKMER. Right. Thank you.

Mr. VOLKMER. Dr. Orville G. Bentley from the USDA, and he is accompanied by—Dr. Bentley, by the way, is Assistant Secretary for Science and Education. He is accompanied by Alan Tracy, Acting Assistant Secretary for Marketing and Inspection Services. And Dr. Bentley has appeared more before this Ag Committee but also before this subcommittee before, and I am sure quite familiar with proceedings. But just for the record, Dr. Bentley, your statement will be made a part of the record in full and you may either review it in full or summarize, however you so desire.

You may proceed.

STATEMENT OF DR. ORVILLE G. BENTLEY, ASSISTANT SECRETARY FOR SCIENCE AND EDUCATION, ACCOMPANIED BY ALAN TRACY, ACTING ASSISTANT SECRETARY, MARKETING AND INSPECTION SERVICES; AND THOMAS M. WALSH, ASSISTANT GENERAL COUNSEL, OGC, USDA

Dr. BENTLEY. Thank you, Mr. Chairman. I am Orville Bentley, Assistant Secretary for Science and Education. And we as a—and we want to express first the appreciation to you and the members of your committee for the opportunity to discuss with you the USDA's granting of the veterinary biological product license for pseudorabies vaccine, a modified-live virus issued to TechAmerica Group, Inc.

I have with me today Mr. Alan Tracy, the Acting Assistant Secretary for Marketing and Inspection Services; Mr. Tom Walsh, the assistant general counsel, dealing with the Marketing Division.

We would like to present our statement, and since it is rather broad, I would like to cover several items of it, with your permission, Mr. Chairman, for the—my hearing this morning. Obviously, we will be pleased to answer questions at the end of our presentation. And I also want to make the point that this statement is being presented on behalf of the Department as a whole.

As has been said several times this morning already, the development of policies, and especially the USDA's policies on biotechnology, is a part of a governmentwide effort to provide a coordinated framework for regulating new technology. And I want to compliment you, Mr. Chairman, and members of your committee for your interest in the development of products derived from biotechnology and their safe and expedient use and development for commercialization.

The Department shares this view and it is in this context and in this spirit that I present our statement this morning.

In response to the anticipated growth in the commercial application of genetic engineering technology, the Office of Science and Technology Policy, or OSTP, in the Executive Office of the President published the "Proposal for a Coordinated Framework for Regulation of Biotechnology" on December 31, 1984.

The OSTP proposal included policy statements by the FDA, the Environmental Protection Agency [EPA], and USDA. As a part of its policy perspective, the USDA believes that agriculture and forest products developed by biotechnology will not differ fundamentally from conventionally produced products and that the existing—existing regulatory framework is adequate to regulate bio-

technology. USDA also has pledged to amend its regulations if developments indicated a need for additional oversight.

In the months since the OSTP proposal was published, USDA has attempted to encourage promising developments in agricultural biotechnology, in both USDA-funded research projects and commercial product development, while protecting human health and the environment through the administration of existing statutes. We have taken a number of steps to put our biotechnology policies into effect.

First, I am pleased to report to this committee that the Secretary has approved the establishment of an Office of Agriculture Biotechnology which will have primary responsibility for implementing and coordinating the Department's policies and procedures relating to all facets of biotechnology.

This includes the conduct of laboratory and field research, experimentation on biotechnological products prior to their commercialization, and all matters of oversight of biotechnology in agriculture.

The new office will report to the Assistant Secretary for Science and Education and perform a staff role for the interagency Committee on Biotechnology in Agriculture which will be known as the Agricultural Biotechnology and Recombinant DNA Advisory Committee [ABRAC], and for a National Biological Impact Assessment Program.

The Assistant Secretary for Marketing and Inspection Services will continue to exercise the Department's existing regulatory responsibilities for products developed through research.

Your first question concerned APHIS' development of guidelines for licensing veterinary biologics derived by recombinant DNA techniques, and the evaluation of those guidelines from USDA's Federal Register Notice of December 31, 1984.

In July 19—in a July 19, 1985, Federal Register Notice, the Secretary of Agriculture delegated authority for coordinating the development and the carrying out of all matters and functions relating to the Department's regulation of biotechnology to the Assistant Secretary for Marketing and Inspection Services.

These responsibilities were further delegated to the Administrator of APHIS. In this same notice the Secretary of Agriculture delegated responsibility for coordinating the development and the carrying out of all matters relating to USDA-funded agricultural research in biotechnology to the Assistant Secretary for Science and Education.

An understanding of the way in which USDA has divided these responsibilities should prove useful to those in the private sector seeking review and/or approval of biotechnology applications. The Assistant Secretary for Science and Education oversees the research activities for the Agricultural Research Service, the Cooperative State Research Service, and the Extension Service, and the Office of Grants and Program Systems.

The Assistant Secretary for Marketing and Inspection Services oversees the regulatory activities of APHIS, which includes the Veterinary Services, the Plant Protection and Quarantine, the Agricultural Marketing Service, and the Food Safety and Inspection Service.

The USDA-funded research projects involving genetic engineering are currently overseen by the USDA ARRC. The applications for veterinary biologic licenses and permits and permit applications for plant pest movements are regulated by APHIS. In the performance of these research and regulatory activities, there will be close coordination between the research and the regulatory agencies in all cases involving genetically engineered organisms.

The Administrator of APHIS established a Biotechnology and Environmental Coordinating Staff—Coordination Staff—to assist him in fulfilling his regulatory responsibilities for biotechnology. This staff coordinates biotechnology regulatory initiatives. The staff serves as a liaison between APHIS and other USDA agencies as well as other Federal agencies.

On September 23, 1985, APHIS published guidelines in the Federal Register for the control and protection of documents containing confidential business information, or CBI, concerning biotechnology. The protection of CBI is understandably a concern of industry, and a protection of trade secrets was urged by numerous commentators representing biotechnology companies. The USDA guidelines are based upon the confidential business information exception contained in the Freedom of Information Act.

USDA has prepared a departmental policy statement on research and regulation of biotechnology for publication in the Federal Register. Similar statements are being prepared by FDA, EPA, NIH, and OSHA as a part of a coordinated policy statement that refines and expands upon the December 1984 notice. The USDA policy statement will also describe proposed guidelines for oversight and safe conduct of federally funded agricultural biotechnology research.

The USDA policy statement includes a description of proposed new regulations under the authority of the Federal Plant Pest Act and the Plant Quarantine Act for genetically engineered organisms which are plant pests.

Also explained in the USDA policy statement are the procedures APHIS follows in licensing veterinary biologics regulated under the Virus-Serum-Toxin Act which may be derived by recombinant DNA techniques or developed from hybridomas.

Among these USDA policy initiatives, the procedure for licensing biologic products derived by recombinant DNA pursuant to the activity of the VSTA are topics of concern to this committee.

Under the Virus-Serum-Toxin Act in 1913, the USDA, APHIS and the Veterinary Services, have exercised regulatory authority over all veterinary biologics imported into the United States or shipped or delivered for shipment interstate. Recent amendments contained in the Food Security Act of 1985 have extended this authority to products which are shipped intrastate or exported, and have given the Department additional enforcement mechanisms such as the power to detain and seize products.

For the purposes of licensing, biologics derived by recombinant DNA techniques or developed by hybridomas, may be classified into three broad categories based on biological characteristics of the new products and the safety concerns they present.

The first category includes inactivated recombinant DNA-derived vaccines, bacterins, bacterin-toxoids, virus subunits, and bacterial

subunits. These nonviable or killed products pose no risk to the environment and present no new or unusual safety concerns. Monoclonal antibody products used prophylactically, therapeutically, or as components or diagnostic kits also are included in this category.

The second category includes those products containing live microorganisms that have been modified by the addition or deletion of one or more genes. Deleted genes may code—deleted genes may code for virulence, oncogenicity, enzyme activity, or other biological—biochemical functions. Added genes may result in the expression of new immunizing antigens or production of novel biochemical by-products such as the beta-galactosidase.

Precautions may be—must be exercised to assure that this addition of specific genetic information does not impart increased virulence, pathogenicity, or survival advantages in these organisms which are greater than those found in the natural or wild-type forms.

It is important that genes added do not compromise—the safety characteristics of the organism. In most cases it is expected that they will be improved, and therefore not pose any new threats to humans, or other animal species, or to the environment.

Genetic information to be added must consist of well characterized DNA segments. A comparison is also required to be made between the genetically engineered organisms and the wild-type form which represented—with respect to biochemical pathways—virulence traits, and other factors affecting pathogenicity.

The third category includes products using live vectors to carry recombinant derived foreign genes that code for immunization—immunizing antigens and/or other immune stimulants. Live vectors may carry multiple recombinant-derived foreign genes since they can carry large quantities of new genetic information. They also are efficient in affecting—infecting and immunizing target animal species. These properties, for example, make vaccinia virus recombinants very popular subjects for vaccine development programs.

Characteristics of safety and transmission must be answered fully before questions and concerns dealing with safety to humans, animals, and to release into the environment can be answered and before products can be considered for licensing.

TechAmerica Group, Inc. was issued a license for a genetically engineered thymidine kinase negative, TK-negative, pseudorabies vaccine, which is a second category product because it contains life—live microorganisms that have been modified by the deletion of a gene. The chronology for issuing the license to TechAmerica Group, Inc. has been distributed to this committee. We have also received copies—you have also received copies of the Environmental Assessment prepared by APHIS's Veterinary Services in the licensing of the vaccine.

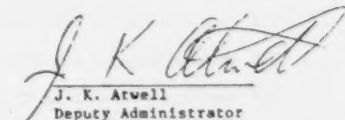
Mr. Chairman, I would ask that—permission to have this statement, the full EA—EA statement inserted as a part of the record. I would ask that consideration.

[The information referred to follows:]

ENVIRONMENTAL ASSESSMENT
AND
FINDING OF NO SIGNIFICANT IMPACT

For the license issued by the United States Department of Agriculture,
Animal and Plant Health Inspection Service, Veterinary Services, under the
Virus-Serum-Toxin Act to TechAmerica Group, Inc., to manufacture, market,
and distribute a recombinant derived pseudorabies virus vaccine for the
purpose of preventing pseudorabies virus infection in swine.

Prepared by
Veterinary Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture


J. K. Atwell
Deputy Administrator
Veterinary Services
Date: April 21, 1986

Environmental Assessment
Recombinant Derived Pseudorabies Vaccine (TK⁻)

TABLE OF CONTENTS

	<u>Page</u>
1. Introduction.....	1
1.1 Finding of No Significant Impact and Summary.....	1
1.2 Background.....	2
1.3 Licensing Procedure.....	4
1.4 Purpose and Need for Recombinant Derived TK ⁻ Pseudorabies Virus Vaccine.....	5
2. The Pseudorabies Virus (PRV).....	6
2.1 Host Range of the Wild-Type Pseudorabies Virus.....	6
2.2 Transmission of Wild-Type PRV to Other Species.....	7
2.3 Pathogenesis of the Wild-Type Pseudorabies Virus.....	9
2.4 Latency and Shedding in the Wild-Type Pseudorabies Virus.....	9
2.5 Reversion of the Wild-Type Pseudorabies Virus.....	10
2.6 Survival and Persistence in the Environment - Wild-Type PRV.....	10
2.7 Pseudorabies Virus (Wild Type) - Persistence in Meat and Animal Products.....	11
2.8 Human Risk of Pseudorabies Infection.....	12
3. Recombinant Derived TK ⁻ Pseudorabies Virus Vaccine.....	14
3.1 Development of Conventionally Prepared Modified Live BUK Vaccine Virus.....	14
3.2 Summary of the Development of Recombinant Derived TK ⁻ PRV.....	14
3.3 Thymidine Kinase: The Critical Enzyme.....	15
3.4 Construction of the Recombinant Derived PRV as Reviewed by APHIS.....	16

4. Safety Issues.....	16
4.1 Pathobiology of the Recombinant Derived PRV.....	16
4.2 Safety Studies Conducted Prior to License Application.....	17
4.2.1 Safety Tests in Mice.....	17
4.2.2 Safety Tests in Rabbits.....	17
4.2.3 Safety Tests in Calves.....	17
4.2.4 Safety Tests in Swine.....	18
4.2.5 Additional Safety Tests in Swine (Maddox Herd).....	18
4.3 Transmission of Recombinant Derived PRV to Other Species.....	18
4.4 Safety Studies Conducted by the License Applicant.....	19
4.4.1 Safety Tests in Mice.....	19
4.4.2 Safety Tests in Sheep.....	19
4.4.3 Safety Tests in Calves.....	19
4.4.4 Other Safety Observations in Swine.....	19
4.4.5 Backpassage Studies.....	20
4.5 Reversion to Virulence.....	21
4.6 Shedding Patterns in Swine.....	22
4.7 Oncogenicity (Induction of Tumors).....	23
4.8 Field Studies in Illinois, Iowa, Minnesota, and Michigan Using Recombinant Derived Vaccine.....	23
5. Conclusion.....	25
6. References.....	26

1. Introduction

1.1. Finding of No Significant Impact and Summary

This document is an Environmental Assessment (EA) and a Finding of No Significant Impact (FONSI). Its purpose is to present and discuss scientific data and other information relevant to licensing of a recombinant derived pseudorabies vaccine, modified live virus, under the Virus-Serum-Toxin Act (21 U.S.C. 151 et seq.) by the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA). Although this document is dated April 21, 1986, environmental concerns were fully identified and reviewed by Department officials prior to licensing the vaccine. This action is intended to provide the general public with documentation of the Department's review and analysis of environmental concerns.

Issuance of a license for a biological product under the Virus-Serum-Toxin Act requires data to establish compliance with APHIS' regulatory requirements of purity, safety, potency, and efficacy. The review for safety of the recombinant derived pseudorabies virus vaccine included an assessment of the impacts of the licensure of the product on the environment. With regard to the impacts of the licensure of TechAmerica's recombinant derived thymidine kinase negative (TK-) pseudorabies virus vaccine on the environment, APHIS found that its action in licensing this vaccine would not have a significant impact on the environment. Such finding is supported by the following facts:

1. Genetic engineering procedures were employed to delete a gene which is essential for virus replication from a strain of conventional modified vaccine virus. The deleted gene coded for the synthesis of an enzyme, thymidine kinase, which is necessary for virus multiplication. Data on file demonstrated that without the enzyme, the virus lacked the ability to multiply in the central nervous system and thus could not establish latency in infected animals. The recombinant derived virus is much safer than naturally occurring or conventional modified vaccine virus.
2. The recombinant derived pseudorabies vaccine virus was rendered essentially noninfectious or avirulent using conventional methodology, i.e., growing the virus in the presence of nucleic acid analogs and naturally selecting the weakened or nonpathogenic virus for additional propagation.
3. Transmission of the recombinant derived vaccine virus could not be demonstrated in backpassage studies using susceptible swine, the virus' primary host.
4. Since the recombinant derived vaccine virus will not replicate in nerve cells, due to its lack of thymidine kinase, its use can help to control the shed and spread of the virulent field strains of the virus from apparently healthy animals. Its use will reduce the dissemination of the virulent virus into the environment.
5. Data established that the genetically engineered gene deletion is a

stable characteristic of the vaccine virus with an extremely low probability of reversion. In the rare event that the virus were to reacquire the thymidine kinase gene from a host-cell, it could regain latency characteristics, but would still be avirulent in swine and unable to spread into the environment.

6. The wild type pseudorabies virus is found widely distributed in nature and it does not contain an oncogene or cancer-causing substance. Because the recombinant derived virus does not contain any new genetic information or other substance and because it is weakened and cannot persist very long in infected cells, there is no likelihood of it being oncogenic.

7. The recombinant derived vaccine virus has been shown to have a reduced virulence for other animal species.

8. The wild-type pseudorabies virus is not considered pathogenic to man. An attenuated or weakened virus such as that used in conventional vaccines or the recombinant derived strain is also avirulent to man.

9. The recombinant derived vaccine virus has demonstrated an inability to revert to the wild strain of the virus (strain normally found in nature).

This EA and FONSI have been prepared in accordance with (1) the National Environmental Policy Act of 1969 (NEPA) (42 U.S.C. 4331 et seq.); (2) Regulations of the Council on Environmental Quality for Implementing the Procedural Provisions of NEPA (Title 40, Code of Federal Regulations (CFR) Parts 1500-1508); (3) USDA regulations implementing NEPA (7 CFR Part 1b); and (4) APHIS Guidelines Implementing NEPA (44 FR 50381-50384 and 44 FR 51272-51274).

This document presents an in depth discussion of scientific data and other information which was considered by APHIS in its licensing decision regarding the recombinant derived pseudorabies virus vaccine. It describes the disease, the causative agent, conventional methods of treatment and the recombinant derived virus and vaccine produced from it. Included in the document are references where much of the data discussed may be found. APHIS' alternative to licensing the new vaccine would have been to refuse to license the product. The regulation and standards under the Virus-Serum-Toxin Act require that, in order to be licensed, animal vaccines must be pure, safe, potent, and efficacious. The recombinant derived pseudorabies live virus vaccine satisfied these standards.

On January 16, 1986, a license to manufacture, market, and distribute a recombinant derived pseudorabies vaccine for the purpose of preventing pseudorabies infection in swine was issued to TechAmerica Group, Inc.

1.2 Background

In this document there will be reference to three different types or forms of pseudorabies virus: (1) The parental or wild-type virus is that virus which is causing disease in the swine industry today. The virus is characterized by

being virulent or infectious. One isolate of this infectious virus is the naturally occurring Bucharest strain. Unmodified Bucharest (BUK) virus is infectious or pathogenic and may be carried by infected animals as latent virus, much like other herpesviruses. (2) The modified live virus is a form of BUK virus, which has been attenuated or weakened and has lost some of its infectious properties. Prior to the licensing of the recombinant derived vaccine, USDA had issued three licenses for modified live virus vaccines and four licenses for vaccines containing killed or inactivated pseudorabies virus (PRV). Vaccines for Pseudorabies have been in use for many years. In both the wild or parental virus and the modified live vaccines, the virus contains all of the genetic information it requires for self replication. Included is a gene which produces an enzyme, thymidine kinase (TK), which is necessary for the virus to reproduce. (3) The recombinant derived pseudorabies virus was developed using Recombinant deoxyribonucleic acid (DNA) procedures for a further modification of the modified live virus BUK strain of PRV. This resulted in a virus which is essentially noninfectious (avirulent) in swine and other animals. In addition, this recombinant DNA derived virus immunizes or elicits the production of antibodies which protect the animal against the disease. One additional feature of the recombinant DNA derived virus is that a gene that is responsible for the incorporation of thymidine into viral DNA, the thymidine kinase gene, has been deleted from the virus. Throughout this document, the recombinant derived virus will be referred to as TK⁻, as opposed to virus forms containing the gene which will be referred to as TK⁺.

Pseudorabies is a contagious disease of swine and other livestock caused by the virus known as PRV. Swine are the primary host for the organism, and piglets under five weeks of age are most susceptible. In swine, the disease causes respiratory illness and encephalitis (inflammation of the brain) which may lead to death. Other common effects of the disease in swine are abortions, reduced litter size, and slowed growth rates. In other livestock, the disease causes lethal encephalitis. Pseudorabies poses no health risk to humans.

Pseudorabies is responsible for major economic losses to the nation's swine industry. Fatality rates often reach 100 percent in pigs under two weeks of age. Losses to the swine industry as a result of the disease amount to millions of dollars. Pseudorabies has escalated to the point where approximately 6.4 million of the nation's 80 million hogs are affected. In the last decade there has been a ten-fold increase in the prevalence of the disease. A number of reasons for the escalation of pseudorabies have been presented. Some of these included the emergence of more virulent strains of the virus, and a wider dissemination of the disease.

PRV infection may be moderated or prevented by the use of vaccines comprised of either killed or modified live strains of PRV. The modified live vaccines are the most effective. Conventional modified live virus PRV vaccines are produced by multiple passages of the virus in culture cells. During tissue culture passage, the virus is naturally mutated or attenuated by losing some of its infectious properties. One disadvantage of modified live virus vaccine is that the vaccine virus can establish itself in the animal's central nervous system and become latent or inapparent. During latency, animals may test

negative for the disease. At times of stress the virus can become active and begin to replicate. This cycling of the virus can complicate diagnosing the disease status of the herd because it may cause animals to become positive to tests used to detect the presence of wild-type pseudorabies virus. This can also cause problems in sale testing of feeder pigs since passive antibody may persist in offspring. If exposed to wild-type virus, vaccinated animals may also become carriers of the disease and transmit it to other animals.

The recombinant derived pseudorabies virus which was used to produce the new vaccine licensed by APHIS is derived from a virus which is found in nature. This modified virus is already used to make vaccines but has been genetically modified to rid it of undesirable traits. This particular virus, was invented, tested, and patented at the Baylor College of Medicine in Texas. The new virus is avirulent or virtually noninfective and is not capable of becoming latent in nerve cells. Also, it does not revert back to the virulent wild strain. However, it can still immunize animals against the disease. Because it possess these traits, the new vaccine virus is many times safer to the environment and animals than the virus found in nature or in conventional live vaccines.

1.3 Licensing Procedure

An application for a product license for the recombinant derived pseudorabies virus vaccine was submitted to APHIS by TechAmerica Group, Inc., on December 27, 1984. The patent application for the gene deleted virus was filed December 31, 1983, and a patent was granted on April 30, 1985. At the time of the license application the virus had already been developed and tested by the inventor. It included both laboratory and field testing of the virus. Such development and testing was not subject to USDA authority under the Virus-Serum-Toxin Act.

When the firm filed its application for a USDA product license it was not seeking permission to develop and evaluate a genetically altered virus with new properties. Instead, it was seeking to manufacture a product from a well characterized virus which had already undergone substantial research and testing with no recorded adverse reactions.

In licensing the product APHIS followed procedures established by the regulations and standards in 9 CFR 101 et seq. The intent of such regulations and standards is to assure the purity, efficacy, safety, and potency of animal biologics. Prior to continuing with the licensing process, APHIS sought additional data involving safety considerations. Such additional data included information concerning the virus' pathogenicity and immunogenicity, as well as the results of the field trials conducted by the inventors in Texas. After review of this safety data, APHIS proceeded with licensing.

The examination of safety, in the context of the licensing of live viral (or bacterial) vaccines, addresses several questions. Will the vaccine, as it is intended to be administered, be deleterious to the species for which it is intended? Will the vaccine virus, when administered, spread from the vaccinated animal to nonvaccinated animals in contact with that vaccinated

animal? What effect will any spread of vaccine virus have on nontarget animals and on the environment? How long does the virus persist in the environment? How is it spread in the environment?

Once initial safety considerations were resolved, the firm was then allowed to bring the recombinant derived virus into its establishment for the purpose of making the Master Seed from which vaccine would be produced. The Master Seed was then tested and these test results were sent to APHIS for review. Samples of the seed virus were forwarded to the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, for confirmatory testing.

Because the virus was genetically altered, NVSL performed two tests in addition to the tests it normally performs to assure the safety. One test was performed to verify that the virus contained a gene deletion as claimed. The other test confirmed that it was the thymidine kinase gene that was deleted.

When APHIS was satisfied with the Master Seed data, the firm was allowed to produce experimental vaccine lots for testing. After test protocols were filed and approved, tests were allowed in four states, with the concurrence of appropriate state officials. The states were Michigan, Minnesota, Illinois, and Iowa. The first test took place at Michigan State University. It was concerned with the vaccine's safety, efficacy, and adverse reactions. The other tests were concerned mainly with safety. On November 12, 1985, the firm provided additional data on safety tests involving sheep, lambs, and mice. These data were satisfactory and the vaccine was licensed on January 16, 1986.

During the licensing and testing phase the vaccine was subject to review by at least two biosafety committees. The firm had established an Institutional Biosafety Committee (IBC) to review various aspects of the vaccines testing and production. At Michigan State University another committee reviewed the research proposal as well as the testing of the recombinant derived virus. USDA's Recombinant DNA Research Committee (ARRC) was also informally kept advised of the licensing review process. The ARRC was kept informed by one of the reviewers of the vaccine application. This reviewer is a member of the ARRC. The APHIS Biotechnology and Environmental Coordinating Staff was also kept advised of the licensing process.

1.4 Purpose and Need for Recombinant Derived TK⁻ Pseudorabies Virus Vaccine

Conventional vaccines used for controlling pseudorabies have been utilized for many years but are accompanied by problems. Existing vaccines have not been totally successful in controlling the spread of pseudorabies due to a unique feature of PRV and other alpha herpesviruses, their ability to enter into a latent state. As the animal recovers from an infection, the wild-type virus can at times retreat to portions of the nervous system where it becomes quiescent and impervious to the body's immune defenses including those resulting from immunization. This latency may be unexpectedly reactivated resulting in recrudescence (reoccurrence) of disease or in a contagious condition known as the carrier state wherein an infected animal shows no outward symptoms of the disease but can transmit or shed infectious herpesvirus intermittently, causing either infection or antibody formation.

During conventional preparation of modified live PRV vaccines, thymidine kinase negative or TK⁻ virus can be isolated. This is similar to the genetically engineered virus. Without the enzyme which codes for thymidine kinase, the virus is unable to replicate. However, unlike the genetically engineered virus, some of the conventionally derived TK mutants are virulent and some can spontaneously revert to TK⁺ at a frequency of one in a thousand to one in ten thousand.

Ideally, a virus for a pseudorabies vaccine would be a modified live virus, avirulent but immunogenic, that would not produce latency in nerve cells. Thus the inventors, to eliminate latency and the potential for spontaneous reversion, deleted the functional portion of the TK gene. Drs. Malon and Saul Kit, both of Baylor College of Medicine, Houston, Texas, using genetic engineering procedures, developed such a product and a United States Patent (No. 4,514,497) was issued on April 30, 1985.

In developing the new modified live PRV vaccine, the inventors reduced the virulence of an established modified live vaccine strain, the BUK strain, without impairing its immunogenicity. A deletion mutation was also engineered into the structural portion of the PRV thymidine kinase (TK) gene. This deprived the virus of its ability to replicate in nerve cells.

The vaccine produced from new recombinant derived PRV is far safer than conventionally prepared products and poses no increased risks to any species of animal.

2. The Pseudorabies Virus (PRV)

2.1 Host Range of the Wild-Type Pseudorabies Virus

Natural infection with wild-type PRV occurs in swine, cattle, dogs, cats, and sheep as well as in several species of wild animals.² A wide variety of additional species has been shown to be susceptible to the disease by experimental inoculation. Studies by a number of laboratories with various domestic and wildlife species all indicate the pig is the only significant maintenance host or carrier. All other species, except for perhaps sheep on a short-term basis, are considered "dead end" hosts. Pseudorabies virus causes a rapidly and acutely fatal infection in most nonswine species.^{1,3,4} After the animal dies, the virus is eventually destroyed and becomes noninfectious. Animals have varying degrees of resistance to the virus as shown by the following chart.

Resistance of Animal Species to PRV Infection

	<u>Resistance to Infection From Wild-Type Virus</u>	<u>Outcome of Infection</u>
Pig	Low	Variable*
Dog	High	Death

Cat	High	Death
Cattle	Moderate	Death (usually)
Sheep	Low	Death (usually)
Raccoon	Moderate	Death
Opposum	High	Death
Rat	High	Death
House	High	Death
Skunk	High	Death

*Depends on age of pigs at time of infection

2.2 Transmission of Wild-Type PRV to Other Species

The wild type PRV has been isolated from naturally occurring infections in wolves, coyotes, foxes, raccoons, Norway rats, mice, mink, skunks, opossums, and roe deer.^{4,5,8,10} Experimental infection has been accomplished in several species of fox, skunk, muskrat, woodchuck, badger, wild rabbit, raccoon, opossum, deer, brown bat, buzzard, ferret, jackal,^{1,6,9,11} mink, porcupine, Rhesus monkey, sable, sparrow hawk, and leopard.

The role that wild animals play in the dissemination of pseudorabies has not been affirmatively documented. Circumstantial evidence, observations, and research data presented thus far have not concluded that any wild animals can act as carrier-shedders of the virus.

Natural infection of most wildlife appears to be via the nasal oral route. Carnivores become infected from eating carcasses, aborted fetuses and placentas from infected swine.^{1,5,6,7} Infection of other species may come from eating feedstuffs contaminated with excreted virus or carcasses of small animals dying of the disease. One report says experimental transmission between raccoons occurred,¹¹ but most authors believe infected swine are the sole source of infection in wildlife.^{1,6,7} One author theorized that rats became resistant and harbored the virus. Another⁴ reported that rats excreted virus for 140 days and that certain ground squirrels harbored the virus for 160-290 days. However, attempts to infect reptiles and insects have not been successful.²

Much more data has been presented on the part rodents play in the transmission of the wild type virus. However, the weight of evidence suggests that rats do not have a significant role as either a reservoir or disseminator of PRV. Rats are a thousand times more resistant to this virus than sheep, yet when they do become infected, they invariably die. Those rats which are exposed but not infected have not been found to develop antibodies or to spread the disease laterally. They may remain susceptible to infection.

Other wild animals in which natural cases have been reported have invariably had fatal courses of infection. Raccoons have been scrutinized more thoroughly than other animals because of their scavenging characteristics and common presence as nocturnal visitors to hog lots and farm building areas. Experimental infections of raccoons revealed that they live from 4 to 6 days after infection but suffer an invariably fatal outcome. Raccoons not

exposed to the virus, but in the same room with infected animals remained uninfected and susceptible to the virus during the course of the trials. However, infection was transmissible from raccoons to swine and from swine back to raccoons. The limited information available concerning other rodent infections suggests patterns similar to those presented. The virus is present in infected animals during the course of the syndrome and is released in the saliva and nasal discharges, this possibly accounts for the low measure of aerosolization of virus. The contamination of feed by saliva from infected animals and the possibility of swine or other animals consuming meat from infected carcasses also constitutes a hazard. The virus has not, however, been isolated from tissues or excreta of feral animals live-trapped on farms where pseudorabies has been present in swine.

Most common economically important domestic animals are susceptible to pseudorabies. Sheep, goats, cats, and dogs all suffer fatal infections from this disease. Reports of recovery with antibody development, have not been found. The same is essentially true in infections of cattle; however, there have been two reported instances of recovery of cattle from pseudorabies.

Infections of dogs and cats in all instances have been reported to be caused by the ingestion of virus-contaminated material, which usually was porcine flesh. Lateral spread among dogs and cats has not been noted, although it seems quite possible because of the presence of the virus in nasal and oral secretions of infected animals and the excessive salivation that is part of the syndrome. Lateral spread has been observed in herds of cattle and flocks of sheep, presumably through aerosols and ingestion of contaminated feed. Horses seem to be infected only rarely and appear to be rather resistant to the virus. Infection among domestic animals occurs through their association with swine.

Many kinds of birds have been found to be susceptible to the wild type PRV under experimental conditions, but reports of natural infections have not been found. Producers have testified on occasion to finding dead birds during an episode of pseudorabies in their swine. Day-old chicks are susceptible to the virus on oral exposure. Seed-eating birds and their young have apparently not been sufficiently investigated to evaluate them as infected shedders or passive vectors of the wild type virus. The susceptibility of birds to vaccine strains either conventional or recombinant derived PRV has not been demonstrated. No cases of transmission, infection, or disease in birds have been reported to be associated with the use of live vaccine strains of PRV.

Reports of infection of human beings have not been found except for one more than 40 years ago. Otherwise, laboratory workers, animal care personnel, and livestock producers who have had contact with infected swine and been exposed to aerosols of the virus have remained asymptomatic and have not developed neutralizing antibodies in their serums. While chimpanzees and Barbary apes appear to be immune two other subhuman primates, rhesus monkeys and marmosets, are susceptible. The threshold of infection for humans is evidently very high via the routes of exposure suggested by the experience of those who have had contact with infected animals.

2.3 Pathogenesis of the Wild-Type Pseudorabies Virus

Evidence indicates that the natural means of infection of swine by wild-type PRV occurs by introduction into the nasal passages, by inhalation, and by ingestion. The primary site of viral replication is in the upper respiratory tract. Studies of the mode of spread of virus by neural routes suggest strongly that the principal pathway is the nerve fibers. It is believed that the virus invades the cells of the nasopharynx (upper respiratory tract). With a lag time of about 5 hours it is possible for replication and release of virus to occur through invasion of the bipolar olfactory cells (sensory cells for smell) and progressive transmission through neurological pathways. At the same time, nerve endings in the nose and mouth provide entry points for virus to be rather quickly transported to the gasserian ganglion at the pons (nerve cells in the lower part of the brain) where an intense ganglioneuritis (inflammation of nerve tissue) develops. Similarly, from nerve endings in the tongue, the virus travels to other nerve cells progressing to the central nervous system. The infection spreads to higher brain cells from these points of entry. At the same time, an infection of secondary importance occurs in the lining of the nose and pharynx, the large air passages, and in the terminal air sacs--in the lungs. Viremia is of low titer (containing a small amount of virus) and is difficult to demonstrate. Experimental evidence further indicates that it is intermittent. However, it provides the virus access to all parts of the body by its spread in this fashion. There are often viral invasions of cells of other organs and the lymphatic system. These invasions do not appear to cause life-threatening damage, however.

Of significant secondary importance is the finding of inflammation of nerve tissue. Death of nerve cells contributes to the early loss of crucial functions and therefore is likely to hasten death in severely affected animals.

2.4 Latency and Shedding in the Wild-Type Pseudorabies Virus

Latent infections by the wild type PRV in swine remain largely a mystery as to conditions of establishment, rate of occurrence, and conditions for reappearance or recrudescence. However, it is known that latent infections from wild type PRV occur in a large percentage of infected swine and that latently infected pigs may shed the virus sporadically for short periods of time. Furthermore, virus excretion by latently infected swine may be induced by stress-related factors, or by experimental treatment with corticosteroids.^{1,2,6}

Information which is not known about the wild type PRV at this time concerns:

1. The percentage of infections which become latent. This is a topic of much controversy. Evidence is mounting that most, if not all, infections develop into latent phase.
2. The percentage of latently infected animals that eventually shed PRV. Current evidence indicates that under normal conditions of hog management,

very few (approximately 1 percent or less) animals shed virus following latent infections.

3. Those factors (environmental and physiological) which may induce shedding, such as farrowing stress.

Persistent wild type PRV infections which exist are of two types; those in which virus can be isolated consistently over a long period (chronic persistence); and those in which virus cannot be isolated by conventional means (latent persistence). Virus has been recovered in chronic infections over long periods. In one study, vaccinated swine surviving natural challenge yielded the virulent strain for as long as 11 months in 2 to 24 percent of animals tested. In another study, virus was recovered from tonsil explants as long as 6 months after infection was observed.^{3,4,5}

2.5 Reversion of the Wild-Type Pseudorabies Virus

Under experimental conditions allowing for rapid passage of the virus in highly susceptible young pigs, the wild type virus was able to increase in virulence. Similarly, the husbandry conditions of commercial production methods also influence incidence and severity of disease episodes. A continuous, open-end enterprise--adding new animals to replace those sold--operates to maintain the presence of the virus on the premises. Similarly, episodes of the disease in herds that are "farrow to finish" in confinement have been noted to result generally in greater losses than episodes wherein pigs are farrowed on pasture and raised in pens in wider physical distribution. The stress of being moved, inclement weather, and mixing with other swine, are believed to be factors which further accelerate the course of the disease and reduce resistance to PRV.⁴

2.6 Survival and Persistence in the Environment - Wild Type PRV

Pseudorabies virus found in nature is a relatively stable virus. It may persist in the external environment for several days to a few weeks.² One study indicated that airborne spread was limited to animals closely associated with virus shedders and that after 2 weeks, contaminated stalls were no longer infective.⁴

Persistence is associated with how well the virus is protected from heat, drying, sunlight and changes in pH. In most cases, virus at infective levels will not persist for many days.³

One study performed on the wild-type virus showed that it persisted for less than 1 week in neutral well water and less than 1 day in chlorinated water.⁴ The virus persists for a longer time in straw than on grass or alfalfa hay. It can survive on contaminated straw and feeding troughs for more than ten days at 24°C (75°F) and for as long as 30 days at 18°C (65°F). The virus may persist for several days on clothing and footwear if kept moist. The virus persists in manure and lagoons for up to two days and may survive in urine free of fecal matter for longer periods.

The virus can persist in carcasses for up to five weeks, and on contaminated wooden boards for seven weeks.^{3,4}

Studies performed at Iowa State University gave the following conclusions concerning the survival of wild-type PRV in the environment.^{1,2}

1. PRV is very susceptible to pH levels below 4 and above 9.
2. Survival is temperature dependent. Higher temperatures kill the virus more rapidly.
3. PRV is killed in hours on clean concrete.
4. PRV will survive up to 3 days on plastic and steel.
5. PRV will survive in a ground corn mixture for only a few hours.
6. PRV will survive in a whole corn for up to 4 days.
7. PRV survives in soil for up to 6 days.
8. PRV survives in lagoon water for up to 2 days.
9. PRV survives in manure for up to 2 days.
10. PRV survives in urine for up to 2 days.

If optimal pH and temperature conditions exist, some virus may survive 40 days at 37° C and 120 days at 4° C. However, these conditions are unlikely to exist in hog raising facilities, and due to that fact the survival of virus in infectious dosages is likely to be very limited indeed. In most cases, virus probably survives only a few days.

Carcasses of pigs or wild animals may contain virus for at least one week under summer conditions.^{2,5} Since protein protects the virus, important potential sources are dead baby pigs, infected placentas and dead wildlife.

2.7 Pseudorabies Virus (Wild Type)-Persistence in Meat and Animal Products

Various groups have studied the wild-type PRV and its persistence in products and by-products of animal origin.¹⁻⁶

In natural cases of pseudorabies, the virus is mostly found in the in the nasopharynx, regional lymph nodes, central nervous system, trachea, and lungs. Experimentally, the virus has been found infectious by all routes. Under natural conditions, the most common route of entry and spread is thought to be the nasal cavity.

It appears that the presence of the virus in the various organs depends on the stage of the disease. Initially, the virus is found in the tonsils, there appears to then be a high concentration in the blood followed by concentration

in the various organs, muscle, skin, and nerves. Virus has also been isolated from urine and feces. Slaughtering of pigs may be the source of slaughterhouse contamination.

European Scientists isolated slaughterhouse strains of the wild type PRV that were pathogenic for both rabbits and foxes. Other investigations have reported deaths in fur animals after ingesting contaminated pig meat.

New Zealand scientists injected wild-type PRV into the hindquarter of a freshly killed pig and evaluated the effects of freezing and time on viral inactivation. They found that the viral inactivation curve is biphasic (has two peaks), with most of the virus being rapidly inactivated, but with a small population of more stable virus persisting for a longer period of time.

In studies of pigs administered wild-type virus by nasal aerosol, the muscles were shown to have significant levels of the virus for at least 30 days after slaughter. These studies suggest that the refrigeration of meats and offal for 30 days at 2°C is not capable of freeing carcasses or offal from the PRV.

The available evidence indicates that viruses contaminating foodstuffs will remain infective for a longer period of time than the foodstuff remains edible. The same is thought true whether the foodstuff is refrigerated or frozen.

The following meat processing methods are reported to promote virus persistence in meat and in animal products:

- Refrigeration
- Freezing
- Salting
- Pickling
- Drying

Although PRV may survive in meat and animal products, this does not cause public health concerns because pseudorabies is not infectious to humans.

2.8 Human Risk of Pseudorabies Virus Infection

The Centers for Disease Control has recorded only one human case with clinical symptomatology of pseudorabies. Attempts by the Centers to isolate virus in specimens submitted from this case were negative. Further, serum neutralizing antibodies could not be detected in serum taken from this patient. At the time of this investigation, the Centers did a search of the American literature and could not find any reports on naturally acquired cases of human pseudorabies that could be verified either by virus isolation or detection of serum neutralizing antibodies. No other cases have been reported to the Centers. According to the Centers, there is no evidence that the PRV poses a health risk to humans. There is also no evidence that it will infect and cause illness in humans. Accordingly, it may be concluded that this is not a disease of public health importance.

Studies have been conducted on the epidemiology of human susceptibility of man to PRV.⁵ The sera of 445 persons of infection-endangered vocational groups or symptomatically suspicious patients were studied for serum neutralizing antibodies against the PRV. Sera were examined from 223 slaughterhouse workers; 107 swine farmers; 5 animal husbandrymen; 7 cow milkers; 2 agriculture students; 2 veterinary swine practitioners; 34 laboratory personnel who routinely work with virus infected material; and 63 symptomatic clinically suspicious patients from rural populations (farmers). Serum neutralizing antibodies could not be detected. The authors report on deliberate attempts by laboratory workers to infect themselves with virus isolated from clinical specimens. The laboratory workers inoculated themselves with $10^{3.4}$ to $10^{6.1}$ ID₅₀ of freshly isolated swine virulent virus. There were no generalized manifestations of disease. Rabbits that were simultaneously inoculated with the same doses died with clinical signs of pseudorabies. Critically evaluating the literature on alleged laboratory infections, the authors concluded that man is not susceptible to PRV infection.

According to Tuncman, humans are slightly susceptible to infection. Tuncman (1938) described three cases of infection of laboratory workers from which the virus was isolated.⁶ The author also reported that in a study of human sera, low titers of antibodies were ascertained. However, Gustafson, a noted expert in swine diseases, reviewed the report by Tuncman and believes there are reasons for doubt. It is the only report found in which gross assumptions were supported by an effort to isolate the virus. In retrospect, it is believed that many of the symptoms manifested by the laboratory workers were psychologically induced given the circumstances. Further, there was no data on virus neutralizing antibodies present in the subjects upon recovery from their alleged infections. More recently, Gustafson examined sera from 16 farmers in an endemic area and sera from workers in his laboratory, including his own serum.⁴ One of the farmers had been bitten on the thumb by a pig from which virus was isolated from the salivary gland. In all cases, tests for serum neutralizing antibodies were negative.²

Data indicate the threshold for human infection is very high. The constant exposure of slaughterhouse workers, pork producers, and laboratory workers, has resulted in no documented cases of human pseudorabies disease. It is known that the respiratory (aerosol) route is the most common method of spread of the virus. In general, laboratory workers do not take any unusual precautionary methods other than good laboratory procedures when handling PRV infected materials. In some states it is not unlawful, and is a common practice for farmers, to slaughter exposed animals for consumption. Federal regulations prohibit the interstate movement of animals exposed to clinical cases for a period of 10 days. However, this is to prevent spread among animals and not for any consideration of human risk. Meat inspection regulations have no special provisions for handling known exposed herds at slaughter. Studies have shown that meat and meat by-products contain live virus. Yet there are no documented or confirmed cases of pseudorabies in humans. This confirms the position of the Centers for Disease Control and the opinions of many veterinary microbiologists that the PRV is of no public

health risk to humans. Disease is confined to susceptible wild and domestic animals. Considering the characteristics of the recombinant derived TK vaccine virus, to produce only transient presence of virus in animals and its lack of infectivity even in swine, the potential for infecting humans does not exist.

3. Recombinant Derived TK⁻ Pseudorabies Virus Vaccine

3.1 Development of Conventionally Prepared Modified Live BUK Vaccine Virus

Conventionally prepared vaccines presently licensed by USDA are developed from mutations of the naturally occurring virus. Field isolates, or viruses found in nature, are extremely variable in pathogenicity (infectivity) and therefore unpredictable.

Most conventional vaccine strains have been genetically altered by passage (growth) of viruses in chicken embryo or cell cultures, or both. The BUK strain of PRV from which Dr. Kit prepared his vaccine virus was isolated and attenuated by 100 passages in chicken embryo and approximately 600 passages in chicken embryo fibroblast cell culture.^{2,3}

These alterations of the virus resulted in gradual reduction in species affected and severity of the disease. The modified BUK Strain isolates currently used in USDA licensed modified live virus vaccines are avirulent for pigs of any age. Pathogenicity for domestic ruminants, mink, ferret, guinea pig, and rabbit is variable.

The Bucharest strain of virus at the 624th passage in cell culture, which approximates the passage level of the virus adapted by Kit, was inoculated into swine fetuses within the uterus. Most inoculated fetuses died, but noninoculated contact fetuses were not affected.¹ We also note that this conventionally-prepared modified live virus can still become latent in the nerve cells of infected swine.

Spread of the conventional modified pseudorabies vaccine virus from swine vaccinated with USDA-licensed modified live virus vaccines has not been demonstrated in susceptible contacts in laboratory trials using either virus recovery techniques or serum neutralization response. Over 23 million doses of these vaccines have been marketed in the last 7 years.

3.2 Summary of the Development of Recombinant Derived PRV TK⁻

The recombinant derived TK⁻ PRV was first developed and patented by Drs. Malon and Saul Kit in 1985 and later sold to TechAmerica Group, Inc., for USDA vaccine licensure.

The inventors employed a conventional modified strain of PRV, the BUK strain, and modified it further by growing the virus in the presence of nucleotide analogs or mutagens (as used to prepare conventionally attenuated live virus vaccines). In separate experiments, the gene coding for the enzyme TK was

deleted from the DNA of the modified BUK PRV thus producing a virus with DNA unable to code for TK. Cells were then transfected with both the modified or mutagen propagated BUK PRV and the TK gene deleted DNA, also from the modified BUK PRV. Procedures were then used to select a BUK PRV that was avirulent or noninfectious, immunogenic and TK⁻. The virus selected was used by TechAmerica to produce a master seed of virus upon which the licensing application was based.

3.3 Thymidine Kinase: The Critical Enzyme

In order to produce a pseudorabies seed virus which subsequently would be used as master seed stock (for vaccine production) characterized as being safer or more effective, the gene coding for the enzyme thymidine kinase was deleted from the viral DNA. Thymidine kinase is important to the life cycle of the PRV. Upon infection of permissive cells, PRV induces the synthesis of virus-encoded enzymes which are essential to maintain the viral life cycle and are necessary for the virus to survive.

Thymidine kinase is one of the enzymes coded for by the genome of PRV and other alpha herpesvirus such as herpes simplex virus (HSV) and infectious bovine rhinotracheitis. The function of TK is to catalyze thymidine through a series of biochemical steps (described in the patent) to ultimately produce viral DNA. Without the enzyme, the virus cannot replicate and survive.

Most tissues of the host produce sufficient amounts of TK within their cells (endogenous cellular TK) to allow for the synthesis and replication of the TK⁻ PRV. However, nondividing tissues, such as nerve tissues, produce only low levels of endogenous cell TK. These cells can support efficient viral DNA synthesis and virus replication only if the gene coding for TK is provided by the infecting virus. If the cell provides TK by making it from cellular genetic material, the infecting virus may utilize this enzyme to make new viral DNA.

The pivotal role of the viral encoded TK in supporting infection of nerve tissues and the establishment of latent infection in ganglia has been demonstrated in studies with HSV. These studies have shown that TK⁻ herpes simplex virus type 1 and 2 (HSV-1, HSV-2) mutants are much less virulent than TK⁺ parental virus strains following either intracerebral or peripheral inoculation in mice and guinea pigs.³ Although TK⁻ HSV mutants found in nature can migrate to sensory ganglia, they replicate poorly in the ganglia and they are much less likely to become reactivated under the conditions used to reactivate latent infections by TK⁺ HSV strains.⁴

Similar information has been developed by Dr. Eit et al. in their studies of TK⁻ PRV.¹ These studies have demonstrated that even after treatment with dexamethasone (a steroid hormone used to induce relapsing infection by the latent virus) PRV was not isolated from nasal swabs, tonsillar scrapings, tonsillar tissue, and trigeminal nerve ganglia of swine vaccinated with his TK⁻ virus.

3.4 Construction of the Recombinant Derived PRV as Reviewed by APHIS

The process for preparation of a PRV which fails to produce any functional TK as a result of a deletion in the TK gene, was submitted to USDA in the license application of TechAmerica. In licensing the production of the vaccine, the following technical details were reviewed:

- (1) Construction of the hybrid plasmid. This plasmid consisted of a cloning vector and a DNA fragment of PRV which contained substantially all of the PRV TK gene;
- (2) Infection of the TK⁺ host cells with the hybrid plasmid of step (1) along with DNA from a temperature-resistant PRV TK⁻ mutagen-induced variant virus;
- (3) Selection in TK⁻ host cells, for PRV TK⁺ from the virus produced in step (2);
- (4) Deletion of DNA sequences from the hybrid plasmid of step (1) such that no functional enzyme in the PRV TK gene remained, while retaining PRV DNA sequences adjacent to each side of the gene deletion;
- (5) Infection of TK⁺ host cells with PRV TK⁺ DNA derived from the PRV TK⁺ obtained in step (3) with the resulting hybrid plasmid of step (4); and
- (6) Selection in TK⁺ host cells, for PRV TK⁻ from the virus produced. In step (5) to produce the PRV TK⁻ deletion mutants, the recombinant derived vaccine virus was developed by this procedure which employed traditional as well as genetic engineering techniques to prepare a stable virus product.

4. Safety Issues

4.1 Pathobiology of the Recombinant Derived PRV

Virologists generally agree that several genes contribute to the ability of alpha herpesviruses to cause disease and, accordingly, that virulence is multigenic or caused by the interaction of many genes. The TK gene is one of the genes which contributes to the virulence of PRV. In other words, the conventional modified live BUK & Bartha strains of PRV are attenuated for virulence compared to wild type field isolates of PRV. Each of these conventional modified live virus strains are PRV TK⁺. The modified live PRV strain, used for the isolation of the recombinant derived TK⁻ virus was a TK⁺ strain previously attenuated by many passages in chick cells. This conventional modified live PRV has been useful for several years in protecting swine against Pseudorabies. It has also been shown to be partially attenuated for cattle, but low doses produce fatal infections in rabbits and mice. Thus, studies in laboratory animals can be used to compare the virulence properties (pathobiology) of PRV types including wild type, modified live, and recombinant.

4.2 Safety Studies Conducted Prior to License Application

Early developmental studies conducted by Dr. Saul Kit and his associates clearly evaluated the safety features of the TK⁻ PRV, as the research progressed from laboratory animals to field studies.^{1,2} These studies which had been developed prior to license application by TechAmerica Group, Inc., were considered as background information in the licensing process, and provided USDA with valuable and extensive safety data. The studies and their significance are briefly summarized as follows.

4.2.1 Safety Tests in Mice

Virulence of the recombinant derived virus was assayed by evaluating mortality patterns in 3-week-old female Swiss mice. The virulence of a virus is usually expressed as the LD₅₀; the dose that will kill 50% of the inoculated animals within a given time. After intraperitoneal inoculation, the LD₅₀ value for conventional modified live PRV, a TK⁺ virus was about 68 pfu/mouse, (plaque forming units per mouse), the recombinant derived TK⁻ virus, had an LD₅₀ value greater than 10⁷ (10,000,000) pfu/mouse. Therefore the recombinant derived virus was approximated 150,000 times less virulent than the conventional modified virus. The recombinant derived TK⁻ virus was also found to be 250 times less virulent than the TK⁺ conventional modified live virus in intracranial inoculation.

Additional studies using subcutaneous inoculation of the recombinant derived TK⁻ showed that mice survived inoculation of 0.1 ml of virus having a titer of 10⁸ pfu/mouse. On the other hand, 10^{5.0} pfu of conventional modified live PRV a TK⁺ virus, killed 4 out of 5 mice indicating a factor of 10,000 times more virulent than the recombinant-derived TK⁻ vaccine virus.

4.2.2 Safety Tests in Rabbits

Additional experiments utilizing rabbits further demonstrated that the LD₅₀ of the recombinant derived TK⁻ virus was much higher than of the conventional modified live virus. These data demonstrate that the recombinant derived virus is much less virulent than conventional modified live virus.

4.2.3 Safety Tests in Calves

Four month old calves were used in this study. In one pen, a calf was inoculated intranasally with 1 ml (2.8x10⁷ pfu/ml) of the recombinant derived TK⁻ virus and was housed with a contact control animal given the same amount of a diluent placebo.

In a second pen, another calf was injected intramuscularly with 2 ml (2.8x10⁸ pfu/ml) of the recombinant derived TK⁻ virus, and a contact control was injected with diluent fluid. Body temperatures remained within normal ranges and there were no clinical signs of disease in any of the calves. No observable differences were detected between responses of principals and contact controls during the 30 day observation period.

4.2.4 Safety Tests in Swine

Three pilot studies were performed with swine. The first pilot study evaluated the safety of the recombinant derived TK⁻ vaccine virus for newborn swine. Three- to 5-day-old piglets were inoculated in the neck muscle with 2 ml (10^7 pfu/ml) of recombinant derived TK⁻ virus. The three piglets were in good health 18 days after inoculation. Second and third pilot studies were performed in collaboration with Dr. Eugene Pirtle of the National Animal Disease Center, Ames, Iowa. In the second study, six 5-week-old pigs were inoculated intramuscularly with 1.5 ml (4.7×10^8 pfu/ml) of the recombinant derived TK⁻ virus. Two other pigs served as controls. Two weeks after inoculation, all pigs were challenged intranasally with virulent wild type PRV. Immunized pigs showed no signs of illness and were killed 10 and 11 days after challenge. No PRV was isolated from the tissues (tonsils and trigeminal ganglia) of any of the immunized pigs.

4.2.5 Additional Safety Tests in Swine (Maddox Herd)

In February 1984, PRV was diagnosed on the Maddox Farm and the herd was quarantined. The threat of continued exposure from possible PRV-carrier feral swine and the likely circulation of PRV in the 1,000 plus grower-finisher pigs near the "clean" breeding herd made immunization worthwhile.

On June 26 and 27, 1984, approximately 1,350 pigs were inoculated intramuscularly with 2 ml (2×10^7 pfu) of the recombinant derived TK⁻ vaccine virus. The study group consisted of more than 700 grower-finisher pigs, 224 nursery pigs, 128 pregnant females, 56 nonpregnant females, 7 boars, and 224 piglets.

With regard to vaccine safety, there were no identifiable adverse reactions in this herd, even though about 1,400 doses of vaccine were administered to susceptible and replacement gilts. Females in all stages of pregnancy, boars and pigs, as young as 1 day were among the vaccinees. No abortions were observed. Litter sizes were normal and the immunization did not increase the average number of stillbirths or mummified pigs. No adverse effects have been noted to date on the reproductive capacity of the sows. Data on pigs immunized between 1 and 3 weeks of age showed that all but 4 of 224 newborns survived and no adverse effects attributable to immunization were observed.

4.3 Transmission of Recombinant Derived PRV to Other Species

The properties of recombinant derived TK⁻ pseudorabies vaccine virus were exhaustively compared with the properties of the wild-type virus. Data gathered from injecting the TK⁻ virus into the most susceptible host animals, piglets, showed that the virus was very difficult to recover from the animals. In two cases where it was recovered, it was TK⁻ and could not be successfully transmitted to other piglets. Therefore, since the recombinant derived vaccine virus does not readily infect its primary host, and does not transmit from vaccinated animals, vaccinated animals cannot serve as the source of infection to other animal species.

4.4 Safety Studies Conducted by the License Applicant

The manufacturer, in this case TechAmerica Group, Inc., U.S. Veterinary Biologics Establishment License No. 272, had to demonstrate that the product made from the recombinant derived TK⁻ Master Seed Virus met applicable USDA standards for licensing set forth in the regulations and that it was pure, safe, potent and efficacious. The vaccine virus that was used in the following animal experiments was derived from recombinant derived TK⁻ PRV stock from Dr. Saul Kit. The virus obtained from Dr. Kit was used to prepare the Master Seed Virus which in turn was used to prepare the recombinant derived PRV vaccine.

4.4.1 Safety Tests in Mice

Additional safety tests in female Swiss mice were conducted by the license applicant essentially as described in safety tests in mice of the recombinant derived vaccine section (4.2.1) earlier in this report. Conventional modified live PRV vaccine had an LD₅₀ in mice of 68, whereas the recombinant derived TK⁻ PRV vaccine virus had 48 LD₅₀ in mice that was more than 10⁶ or 10,000,000. These data demonstrate that the genetically engineered vaccine virus was approximately 130,000 times safer than conventional modified live vaccine strains when tested in laboratory mice.

4.4.2 Safety Tests in Sheep

Six yearling (3-month-old) ewes, serologically negative for PRV, were used. Two sheep were inoculated with conventional modified live PRV and two with the recombinant derived TK⁻ virus. All four animals were inoculated intravenously with about 4x10⁸ pfu/animal. Two sheep were maintained as contact controls.

Sheep vaccinated with the recombinant derived TK⁻ vaccine virus had a fever for 3 days but quickly recovered and showed no other signs of disease. In contrast, sheep vaccinated with the conventional modified live pseudorabies vaccine developed high fevers and died 5-6 days after infection.

On day 30, postvaccination, the control sheep and the two vaccinated survivors were challenged with virulent wild type PRV. The control sheep both died 4-5 days after challenge exposure, whereas the vaccinated sheep remained normal.

4.4.3 Safety Tests in Calves

A further indication that recombinant-derived PRV has reduced virulence came from studies in calves. Calves inoculated with conventional modified live PRV developed clinical disease. Calves inoculated with the recombinant derived TK⁻ vaccine virus, showed no signs of disease.

4.4.4 Other Safety Observations in Swine

Although the following results were taken from studies designed primarily to evaluate the effectiveness of the recombinant derived TK⁻ pseudorabies

vaccine, the results from challenging both vaccinated and control animals offer additional evidence of the recombinant product's safety characteristics.

In a preliminary study conducted at Michigan State University, 23 3-day-old piglets vaccinated with the recombinant derived TK⁻ vaccine along with five unvaccinated controls were challenged with virulent, wild-type PRV 22-26 days following immunization. All 23 vaccinated animals resisted challenge with only transient pyrexia (fever), minor malaise, and without neurologic signs of pseudorabies. All five controls showed multiple signs of pseudorabies including high and persistent pyrexia and severe neurologic signs, and four of the five controls died.

In the final efficacy study, 24 PRV susceptible nursing piglets, were divided into four groups and each group vaccinated with decimal dilutions of the recombinant derived TK⁻ PRV vaccine. All vaccinates as well as eight unvaccinated litter mate controls were challenged with virulent wild-type PRV at three different dose levels. All eight controls showed signs of pseudorabies with persistent high fevers, depression and anorexia. Seven showed central nervous system signs and died between the 3rd and 9th day postchallenge. Pseudorabies challenge virus was isolated at necropsy from all seven nonvaccinated controls. All piglets vaccinated with the recombinant derived TK⁻ PRV vaccine survived the challenge with only transient fever and malaise. Pseudorabies virus was not isolated from the tissues of the 14 vaccinates that were necropsied after euthanasia on the 14th day, meaning that, in this study, the recombinant derived TK⁻ PRV vaccinated animals were protected from infection with the wild strain.

Transient central nervous system signs were seen in only one of the vaccinates challenged at 10 times the control level dose.

4.4.5 Backpassage Studies

Backpassage studies were conducted by TechAmerica Group, Inc. at Michigan State University under USDA licensing procedures. These were important for a determination of the genetic stability of the vaccine virus. The studies were done by administering the vaccine to susceptible piglets less than a week old, then attempting to isolate the virus from nasal secretions after vaccination, and then sequentially backpassing or putting the isolated virus back into other susceptible piglets. Very young piglets are probably the most susceptible hosts.

The results of these studies are summarized as follows:

GROUP A consisted of 4- to 5-day-old piglets, each inoculated intramuscularly with $10^{5.5}$ TCID₅₀ of recombinant derived vaccine virus. Tissue Culture Infective Dose (TCID₅₀) is the amount of virus necessary to infect 50 percent of the tissue culture plates exposed. Nasal swabs were taken daily from day 0 to day 9. Virus could not be isolated and all five piglets responded by 14-21 days postvaccination with PRV neutralizing antibody.

GROUP B consisted of 3- to 5-day-old piglets inoculated intramuscularly, each with $10^{6.3}$ TCID₅₀ of recombinant derived vaccine virus. Of 20 nasal swabs taken between the first day of inoculation and 9 days later, two samples on the 5th day were positive for PRV. The excreted virus was shown to consist of the recombinant derived TK⁻ vaccine strain.

GROUP C - Five more piglets were inoculated intranasally with the virus from the Group B 5th day isolation. Pseudorabies virus could not be detected in the 20 swabs taken between "infection" and 9 days later. All test animals remained sero-negative to PRV antibody.

GROUP D - One final "blind" backpassage was conducted with the positive swabs from Group B piglets by intranasal inoculation into an additional five piglets highly susceptible to infection. PRV was not isolated from these animals and all animals remained sero-negative.

In summary, these studies clearly demonstrate the stability of the vaccine virus and its inability to be transmitted. A low incidence of nasal excretion of recombinant derived TK⁻ deleted PRV occurred after intramuscular inoculation of nursing piglets. The titer was low (less than 60 TCID₅₀) and only TK⁻ virus was excreted. This nasally excreted vaccine virus could not be passaged intranasally to other susceptible piglets. These findings are consistent with the characteristics of the conventional modified live PRV from which it was derived.

4.5 Reversion to Virulence

Recombinant derived TK⁻ was tested for reversion both to virulence and to TK⁺ in backpassage studies, which are among routine prelicensing requirements of USDA in assessing safety and genetic stability of the product. In separate experimental groups, either no virus was isolated, or isolated virus was shown to be identical to the recombinant derived TK⁻ PRV.

Since the vaccine virus lacks the DNA sequence which codes for TK, reversion to TK⁺ does not occur. If the TK⁻ construct used is a complete deletion of the thymidine kinase gene, then the probability of acquiring the thymidine kinase gene from the host or other cellular tissue is nearly zero, i.e., 10^7 (or one in ten million).

The reacquisition of this gene (TK) from the vaccinated host would have to occur by homologous recombination. The probability of this happening is nearly zero since (1) the TK sequence necessary for homologous pairing of cellular and integrated viral DNA (prior to the recombination event) has been removed from the virus and (2) the state of the PRV genome in latently infected tissue is unclear suggesting that PRV may not persist in cells by integrating itself into the cellular genome.¹ In the highly unlikely event that the recombinant derived vaccine virus would acquire the gene which codes for TK⁺ from the cellular DNA, it would be the same as the currently licensed conventional modified live virus vaccines.

It should be noted that each serial of the recombinant derived PRV vaccine is

tested by the manufacturer to demonstrate that the virus is TK⁻. These results are then confirmed by additional testing at USDA's National Veterinary Services Laboratories in Ames, Iowa.

4.6 Shedding Patterns in Swine

Shedding patterns of two virulent, wild-type strains of PRV (P-2208 and KC-152-D) one conventional modified live PRV vaccine strain, and the recombinant derived TK⁻ pseudorabies vaccine strain following intranasal inoculation into feeder pigs were studied and are discussed below.

Intranasal inoculation with 5,000 TCID₅₀ of the wild-type KC-152-D strain or 50,000 TCID₅₀ of the wild-type P-2208 strain resulted in continuous virus shedding in all pigs up to 14 days postinoculation. Some of the pigs further shed virus from the wild-type strains in a continuous or discontinuous pattern up to 19 or 20 days postinoculation. The time of onset or the level of virus neutralizing antibody produced in individual pigs was not found to have an influence on their shedding patterns. Three of four pigs inoculated intranasally with the 10⁶ TCID₅₀ of the wild-type P-2208 strain were still shedding PRV on the 22nd day postinoculation.

Pigs inoculated intranasal with 500 or 5,000 TCID₅₀ of the conventional modified live PRV vaccine did not shed detectable amounts of virus. Shedding of this strain by four pigs inoculated intranasal with 7.2x10⁵ TCID₅₀ was not detected between 3 to 7 days postinoculation.

The 3-5 day old piglets inoculated intramuscularly (IM) or intranasally with 10^{5.3} TCID₅₀ of recombinant derived TK⁻ PRV, did not shed virus. All five pigs in this trial were negative for virus shedding for 9 days postinoculation. Also, all responded by 14-21 days after infection with demonstrable levels of virus neutralizing antibody titers.

When an additional five pigs were inoculated intramuscularly each with 10^{6.3} TCID₅₀ of the recombinant derived TK⁻ PRV two, of the twenty nose swabs taken on the 5th to 9th day of observation, contained virus. The excreted virus collected from nasal swabbing was shown to be only the recombinant derived TK⁻ vaccine strain virus.

Piglets were then inoculated intranasally by swabbing both nostrils with the isolated virus from the two pigs described above. PRV could not be isolated in the 20 swabs taken between "infection" and 9 days later. Additionally, none of these animals developed PRV antibody responses. This finding suggests the absence of replicating PRV.

One final "blind" passage was conducted with the two positive isolations from the nasal collection. An additional five piglets were each inoculated intranasally with this material. PRV could not be reisolated from these piglets and all pigs remained serologically negative through a 21 day observation period.

Thus, based on the study of post vaccination virus excretion as detected in

nasal swabs, only two of ten nursing piglets showed transient vaccine virus excretion on the 5th day after intramuscular inoculation with $10^{6.3}$ TCID₅₀ of the recombinant derived vaccine virus.

4.7 Oncogenicity (Induction of Tumors)

Wild-type PRV, the virus found in nature, is not a tumor virus. There have been no reports of oncogenicity associated with PRV infection in any animal species. The genetic engineering methods for the preparation of recombinant derived TK⁻ PRV do not introduce any new information into the already nontumorigenic PRV virus.

Further, the removal of the gene (TK) which allows the virus to persist in infected cells weakens the virus by reducing and virtually eliminating the possibility of virus latency. Since latency has been associated with oncogenicity, any potential, not yet detected, link between PRV and oncogenicity would be even more remote since the recombinant derived TK⁻ virus is less latent than wild-type virus.¹ What follows is a brief consideration of the elements concerning activation of an oncogene.

Two mechanisms of oncogene activation or derepression may involve either: (1) insertion of an oncogene into the cellular genome where it may become activated or derepressed by flanking cellular sequences, or (2) insertion of a promoter (e.g. long terminal repeats) or enhancer sequence into the cellular genome next to an oncogene. In either case, the information for tumor expression must come from an oncogene. Since pseudorabies viruses do not contain oncogenes, neither wild-type PRV nor the recombinant derived TK⁻ PRV can be considered a potential source of oncogene sequences for transmission to host cells.

In comparing the role of latent PRV as a promoter or derepressor with the recombinant strain, the recombinant derived TK⁻ PRV significantly reduces the incidence of latency compared to animals inoculated with conventional modified live PRV vaccines. There have been no reports of tumors associated with either wild type PRV, conventional modified live PRV, or recombinant derived TK⁻ PRV in infected or immunized animals. Therefore, postulating an activating or derepression mechanism of PRV for tumorigenicity is like exploring a causative mechanism where there is no effect.

4.8 Field Studies in Illinois, Iowa, Minnesota and Michigan Using Recombinant Derived Vaccine

Unlicensed veterinary biological products for experimental purposes may be shipped only when authorized by the Deputy Administrator of Veterinary Services in accordance with the regulations. The Deputy Administrator may authorize such shipment if it is determined that the conditions under which the experiment is to be conducted are adequate to assure safety and prevent spread of disease and if procedures set forth in the request are approved.

Request for authorization to distribute unlicensed veterinary biological products for experimental field studies must be accompanied by:

1. A copy of permit or letter of permission from state authorities of each state involved.
2. A list of the names of the proposed recipients and quantity of experimental product to be shipped to each.
3. A description of the product, recommendations for use, and results of preliminary research work.
4. A copy of labels or label sketches which show the name or identification of the product and bear a statement, "Notice! For Experimental Use Only -- Not For Sale," or the equivalent.
5. A proposed general plan covering the methods and procedures for evaluating the product and for maintaining records of the quantities of experimental product prepared, shipped, and used. (At the conclusion of the field studies, results must be obtained, summarized, and submitted to veterinary services.)
6. Data acceptable to the Deputy Administrator demonstrating that use of the experimental biological product will not likely result in the presence of any unwholesome condition in the edible parts of animals presented for slaughter.
7. A statement from the research investigator or research sponsor agreeing to furnish, upon the Deputy Administrator's request, additional information concerning each group of meat animals involved prior to movement of these animals from the premises where the test is to be conducted.

Through the development, submission, and review of such materials, data, and information before approval, veterinary services is able to protect the livestock population, the environment, and the public health from negative impacts. Participation of state authorities is assured in the approval. The location of each study and the number of animals involved is determined so that if unexpected problems develop, immediate corrective actions can be taken. Review of data required, permits APHIS to evaluate and assess the potential impacts of the experimental product and to adjust the procedures and conditions of the study, as defined in the protocols that are submitted, to assure safety.

This procedure has been successfully applied to the authorization of field trials in the licensing of veterinary biological products for over 25 years and has been fully successful in preventing any negative impacts on the environment or public health.

The safety of the new recombinant derived TK⁻ PRV vaccine was evaluated in piglets under field conditions in four states. The tests were designed to confirm the safety of the product in the host animal when large numbers of animals were exposed to the vaccine. These field studies were a prelicensing requirement and were carried out in accordance with the provisions stated in 9 CFR 103.3. The recombinant derived vaccine used was prelicensing Serial 200-1001, prepared in TechAmerica's USDA-licensed facility in accordance with

a filed Outline of Production as required under USDA regulations and standards for product licensing.

In each state, the study consisted of vaccinating 250 nursing or newly weaned piglets followed by an additional 1,000 doses to be used in breeding animals in one state (Michigan). The recombinant derived PRV vaccine was tested in accordance with stated provisions in Section V of the Outline of Production. This section describes all the test procedures which must be satisfactorily conducted by the manufacturer on every serial of product before it becomes eligible for consideration for release. USDA may conduct confirmatory testing at its National Veterinary Services Laboratories prior to releasing the serial for sale and distribution.

The safety trial protocol, approved by USDA on November 3, 1985, consisted of (1) Observations for immediate postvaccination reactions, (2) A second observation 7-14 days later in which each animal would be re-examined, and (3) The last examination to be conducted 21 days postvaccination.

In the event of serious adverse reaction(s), illness, or death, the protocol provided that attempts would be made to reach a definitive diagnosis at a state diagnostic laboratory or university laboratory by gross necropsy and tissue examination for possible isolation of PRV virus. Arrangements were made for freezing and retaining suspect samples of isolated virus for submission to TechAmerica Group, Inc., for examination of the TK⁻ deletion.

Each cooperator was also made aware that this product may stimulate the production of PRV antibody titers in vaccinates which may persist and jeopardize the pseudorabies antibody-free status of a clean herd.

The case report from all three independent safety trials did not reveal any adverse reactions from the vaccine virus.

5. Conclusion

In conclusion, the new recombinant derived pseudorabies modified live virus vaccine was found to be pure, safe, potent, and efficacious by USDA's Veterinary Services. It was produced according to regulations and standards, and adequately tested. The vaccine is needed for the prevention and control of Pseudorabies. To date, no adverse reactions, or adverse impacts on the environment resulting from the administration of the vaccine have been reported to or observed by USDA's field veterinarians. Data and other information available to the Department, as described in this document, support the finding of no significant impact.

6. REFERENCES

2.1 Host Range of the Wild-Type Pseudorabies Virus

1. Crandell, R.A., R.E. Mock, G.M. Mesfin. Latent pseudorabies virus infection in vaccinated swine-recent findings. Proc. Pig Vet. Soc. Cong., Copenhagen, Denmark, p. 119, 1980.
2. Davies, E.B., G.W. Beran. Spontaneous shedding of pseudorabies virus from a clinically recovered post parturient sow. JAVMA 176: 1345-1347, 1980.
3. McFerran, J.B., C. Dow. The excretion of Aujeszky's disease virus by experimentally infected pigs. Res. Vet. Sci. 5: 404-411, 1964.
4. Sabo, A., J. Rajkoni. Latent pseudorabies virus infection in pigs. Acta. Virol. 20: 208-214, 1976.

2.2 Transmission of Wild-Type PRV to Other Species

1. Arnaudov, H.R. On Some New Aspects of the Epizootiology of the Aujeszky's Disease. Vet. Sharnik, Vol. 68, No. 3, 1971.
2. Gustafson, D.P. Pseudorabies Disease of Swine. 4th Edition, 1975.
3. Ihlenburg, H. and Senf, W. Report on Aujeszky's Disease in Carnivores. Monatsh Veterinarmed, Vol. 22, No. 23.
4. Loukachov, I.I., Nikitini, N.G. Aujeszky's Disease in the USSR. Bul OIE, Vol. 678, No. 11-12, 1968.
5. Lyubashenko, Tyul'panova, A.F., and Grishin, V.M. Experiment of Specific Prophylaxis, Treatment and some Questions of Epizootiology of Aujeszky's Disease in Furry Animals. Veterinariya, Vol. 37, No. 4, 1960.
6. Thawley, D.C. and Wright, J. C. Pseudorabies Virus Infection of Raccoons: A Review. Journal WDA, Vol. 18, No. 1, 1982.
7. Toma, B. Epidemiology of Aujeszky's Disease. Dep. Vet. Q.S. Roger Ballon, Vol. 11, No. 3, 1976.
8. Trainer, D.O. Pseudorabies. Chapter 8.

9. Trainer, D.O., and Karstad, L. Experimental Pseudorabies in Some Wild North American Mammals. *Zo. Res.*, Vol. 2, 1963.
 10. Woods, G.T., Starkey, A.L. and Spencer, F.L. Epidemiology of Pseudorabies in Illinois Swine Herds. I. Results of a Serologic Survey and Reported Virus Isolations in 1976 and 1977. *Bovine Practice*, Vol. 1, No. 6, 1980.
 11. Wright, J.C. and Thawley, D.G. Role of the Raccoon in the Transmission of Pseudorabies: A Field and Laboratory Investigation. *A.J.V.R.*, Vol. 41.
- 2.3 Pathogenesis of the Wild-Type Pseudorabies Virus
1. Gustafson, D.P. Pseudorabies. In *Diseases of Swine*. 5th Edition. Editors: A.D. Leman, R.D. Glock, W.L. Mengeling, R.H.C. Fenney, E. School, and B. Straw. Ames, Iowa State University Press. 1981:209-223
- 2.4 Latency and Shedding in the Wild-Type Pseudorabies Virus
1. Davies, Es.B. and Beran, G.W. Influence of environmental factors upon the survival of Aujeszky's disease virus. *Res. Vet. Sci.* 31, 1981:32-36.
 2. Freund, J.D. The effects of environmental fomites on the survival of pseudorabies virus. M.S. Thesis, Iowa State Univ., 1981.
 3. Mare, C.J. Pseudorabies (Aujeszky's Disease): The virus and its pathogenesis in pregnant swine. In *Proceedings. Symposium on Pseudorabies (Aujeszky's Disease)*. Peoria, Il, May 27, 1975:3-6.1
 4. Mare, C.J. The pseudorabies virus. *Pseudorabies Fact-Finding Conference*. Ames, Iowa State University. April 4-5, 1977:202-206.1
 5. Thawley, D.G. Epidemiology of pseudorabies virus: Current knowledge. In *Pamphlet. Livestock Conservation Institute's Pseudorabies Committee*. 2nd Ed., November 1983:i-15.
 6. Wright, J.C. and Thawley, D.G. Role of the raccoon in the transmission of pseudorabies: A field and laboratory investigation. *Am. J. Vet. Res.* 41, 1981:581-583.

2.5 Reversion of the Wild-Type Pseudorabies Virus

1. Gustafson, D.P. Pseudorabies. In Diseases of Swine. 5th Edition. Editors: A.D. Leman, R.D. Clock, W.L. Mengeling, R.H.C. Penney, E. School, and B. Straw. Ames, Iowa State University Press. 1981:209-223.

2.6 Survival and Persistence in the Environment - Wild-Type PRV

1. Donaldson, A.I., et al. Experimental Aujeszky's Disease in Pigs: Excretion, Survival, and Transmission of the Virus. The Veterinary Record, 1983.
2. Freund, J.D. the effects of environmental fomites on the survival of pseudorabies virus. M.S. Thesis, Iowa State University, 1981.
3. Mare, C.J. Pseudorabies (Aujeszky's Disease): The Virus and its pathogenesis in pregnant swine. In Proceedings. Symposium on Pseudorabies (Aujeszky's Disease). Peoria, IL, May 27, 1975:3-6.
4. Mare, C.J. The pseudorabies virus. Pseudorabies Fact-Finding Conference. Ames, Iowa State University. April 4-5, 1977:202-206.
5. Pseudorabies Committee LCI. Epidemiology of Pseudorabies Virus: Current Knowledge, 1986.11

2.7 Pseudorabies Virus (Wild-Type) Persistence in Meat and Animal Products

1. Danner, D. and A. Mayr. Virus Persistence During the Processing of Foodstuffs of Animal Origin Schlachten and Vermarkten (German) Vol. 80, No. 9.1
2. Davies, E.B., G.W. Beran. Influence of Environmental Factors Upon the Survival of Aujeszky's Disease Virus. Research in Veterinary Science, 1981, 31, 32-36.
3. Durham, P.J.K., A. Cow, and W.S.H. Poole. Survival of Aujeszky's Disease Virus in Frozen Pig Meat. Research in Veterinary Science, 1980, 28, 256-258.
4. Freund, J.D. The Effects of Environmental Fomites on the Survival of Pseudorabies Virus. Graduate Thesis, Iowa State University, Ames, Iowa, 1981.
5. Persistence of Viruses in Products of Animal Origin.

Commission of Eur Comm., CEWC Internal Information Agri (165): 206-222, Dec. 1975.

6. Solomkin, P.S. and M.I. Tutushin. Duration of Preservation of Aujeszky's Disease Virus in Feeding Stuffs and on Animal Care Objects. Veterinayiya (Russian), Vol. 33, No. 4, 1956:49-50.

2.8. Human Risk of Pseudorabies Infection.

1. Gustafson, D.P. Diseases of Swine. 4th Ed.
2. Gustafson, D.P. (Personal communication)
3. Dr. Kenneth Herman, Center for Infectious Diseases, Viral Disease Division, Centers for Disease Control, Atlanta, Georgia
4. Jentsch, K.D. and Apostoloff, A. Human Susceptibility to Herpesvirus Suis. (Translated from German) Journal of Hygiene and Related Subjects. Vol. 16, No. 9. 1970.
5. Kissling, Robert E. Diseases Transmitted From Animals to Man. Edited by W.T. Hubbert, W.F. McCulloch; P.R. Schuurrenberger. 6th Ed.
6. Tuncman, Z.M. Observation of Aujeszky's Disease in Man. (Translated from French) Ann. Inst. Pasteur, 670:55. 1938.

3.1 Development of Conventionally Prepared Modified Live Vaccine Virus

1. Jamrichova, O., et al. Acta Virol., Prague 15, 286-292. 1971.
2. Skoda, et al. Acta Virol., Prague 8, 1-9. 1964.
3. Zuffa, A. and Polak, V. Bull. Off. Int. Epizoot. 64, 297-307. 1965.

3.3 Thymidine Kinase: The Critical Enzyme

1. Kit, S., Kit, M., Pirtle, E.C. Attenuated Properties of Thymidine Kinase-Negative Deletion of Pseudorabies Virus. Am. J. Vet. Res., 1985, 46:1359-1367.
2. Kit, S., H. Qavi, M. Hagen, et al. Biochemical Transformation of LM(TK⁻) Cells by Hybrid Plasmids Containing the Coding Region of the Herpes Simplex Virus Type 1 Thymidine Kinase Gene Virology 113:452-454, 1981.

3. Klein, R.J., the Pathogenesis of Acute, Latent and Recurrent Herpes Virus Infection.
Arch. Virol, 12:25-36, 1983./1
- 3.4 Construction of the Recombinant Derived PRV as Reviewed by APHIS
 1. United States Patent No. 4,514,497.
- 4.2 Safety Studies Conducted Prior to License Application
 1. Kit, S., Kit, M., Pirtle E.C. Attenuated Properties Of Thymidine Kinase-Negative Deletion Mutant Of Pseudorabies Virus.
Am. J. Vet. Res. 1985; 46:1359-1367. See p. 1363. Table 1.
 2. Kit, S., Kit, M., Lawhorn, B., McConnell, S. Immunization Of Pregnant pigs in a Quarantined Swine Herd with a Thymidine Kinase Deletion Mutant Of Pseudorabies Virus. High Technology Route to Virus Vaccines.
Amer. Soc. for Microbiology 1985; 82-99. See p. 91.
- 4.5 Reversion to Virulence
 1. Ben-Porat, T. et al. "Latency of Pseudorabies Virus" in Latent Herpesvirus Infections in Veterinary Medicine, Wittmann, R.M., Gaskill and Reeks, R.J. (Eds.) Martinis Nijohh Publishers, 1984.
 2. Yoskum, G. Laboratory of Human Carcinogenesis, National Cancer Institute, National Institutes of Health
Bethesda, Maryland
- 4.7 Oncogenicity (Induction of Tumors)
 1. Bishop, J.W. Department of Microbiology and Immunology,
University of California Medical Center
San Francisco, California

Dr. BENTLEY. I would also like to discuss a number—a matter of voluntary suspension of sales of OMNIVAC by TechAmerica Group, Inc. The procedures followed by the Department in granting the license was consistent with USDA regulatory procedures. The voluntary suspension was agreed to by the Department and TechAmerica to permit USDA to provide full documentation describing the analytical steps which had been taken to ensure that the product was environmentally safe.

The 2-week expansion—suspension agreed upon by USDA and TechAmerica in no way suggested any lack of confidence by the Department or the manufacturer in the safety and efficacy of this product or in the adequacy of measures taken—measures USDA took to carry out our responsibilities under the Virus-Serum-Toxin Act.

Environmental questions about stability, survival, persistence, and dissemination were identified and resolved by APHIS officials prior to licensing of the vaccine. The EA, now available, fully documents the Department's review and analysis of environmental impacts and supports the Department's licensing of this product.

You asked additional questions concerning the role of Science and Education and ARRC in reviewing requests for field testing genetically engineered organisms.

USDA is in the process of publishing USDA Guidelines for Biotechnology Research that will closely parallel the NIH Guidelines and will be issued under the authority of the Food Security Act of 1985.

The amendment gave the Secretary of Agriculture responsibility for establishing "appropriate controls with respect to the development and use of the application of biotechnology to agriculture."

All federally funded agriculture biotechnology research or agriculture research conducted at an institution receiving USDA funds would be subject to the USDA Guidelines for Biotechnology Research unless specific—the research—the specific research project is supported by and subject to guidelines or regulations of another Federal agency.

These guidelines would encompass all phases of agricultural biotechnology research such as: contained laboratory experiments; specialized isolation research, for example, a greenhouse or a biotron; and research involving release into the environment.

To encourage voluntary compliance from industry, USDA proposes to adopt the NIH policy of providing the researchers not required to comply with these guidelines the opportunity to have their new biotechnology research proposals voluntarily reviewed by USDA.

We have developed a system of oversight of biotechnology research in agriculture based largely on our participation in the NIH/RAC process for the past 10 years. I will propose that the Secretary replace the current Agricultural Recombinant DNA Research Committee with a committee closely patterned after the existing NIH/RAC.

We are in the process of establishing a National Biological Impact Assessment Program to provide support in the evaluation and monitoring of the potential impact of biotechnological processes and products on safety and the environment.

Now more specifically, let me share the following review of genetically engineered products mentioned above. From the earliest concerns about recombinant DNA research, and prior to the Asilomar Conference in 198-75 to discuss these concerns, the USDA has been actively involved. USDA was invited to participate in the Asilomar Conference, and the USDA representative met with the National Institutes of Health officials during the formative stage—stages of their Recombinant DNA Advisory Committee now known as NIH/RAC.

Agricultural scientists have been in attendance and members of the NIH/RAC since its first meeting. Agricultural scientists, including a number—a member of the USDA of RAC—of ARRC, are members of the NIH Plant Working Group.

A major portion of the Agricultural DNA Research Committee's time since the fall of 1985 has been devoted to helping to develop the USDA's statement of policy for Research and Regulation of Biotechnology Processes and Products. This statement of policy has been in response to OSTA—OSTP's effort to provide a coordinated framework for regulation of biotechnology.

ARRC's role in the review of applications to field test genetically engineered products is to provide scientific advice. In the University of California proposal to test—field test a genetically engineered or "ice-minus" bacterium, the NIH/RAC recommended approval for the field test, but only after asking ARRC to also review this request and make a recommendation to it.

The ARRC conducted their own review and recommended to NIH/RAC that the proposed field test be approved. This is the procedure that has been followed by RAC and ARRC in dealing with any agriculturally related request that has come to NIH/RAC.

Current EPA policy states that there must be notification—notified prior—that they must be notified prior to all small scale field tests involving certain microbial pesticides, in order to determine whether experimental use permits are required. This is a change from their earlier policy that prior notification and experimental use permits were not required for any 10-acre or less field tests of microbial pesticides.

Their new policy also emphasizes the inclusion of microorganisms genetically altered or manipulated by humans. With this EPA announced policy and OSTP's efforts over the past couple of years to develop a better coordinated approach to regulation of biotechnology, the ARRC has been invited to send representatives to sit in on several of EPA's advisory panel sessions contributing—considering prior notification of desired small field test—field testing of genetically engineered microbial pesticides.

The ARRC has reviewed only one proposal for field testing of genetically-engineered product since publication of the Department's proposed biotechnology regulation in the Federal Register notice of December 31, and that product was a product produced by Calgene Inc., as to which review is not final. The—the ARRC did not review the field testing of Omnivac. However, such review is not required by the Department's licensing procedures.

I appreciate the opportunity to read the statement into the record. We would be pleased to answer any questions if you have them, Mr. Chairman and members of the committee.

[The prepared statement of Dr. Bentley follows:]

FOR RELEASE BY THE COMMITTEE
ON SCIENCE AND TECHNOLOGY

United States Department of Agriculture

Statement of Orville G. Bentley

Assistant Secretary for Science and Education

Before the House Committee on Science and Technology, April 29, 1986

Subcommittee on Investigations and Oversight

Mr. Chairman and Members of the Committee, we wish to express the appreciation of the U.S. Department of Agriculture (USDA) for the opportunity to discuss with you USDA's granting of a veterinary biological product license for pseudorabies vaccine, modified live virus, issued to TechAmerica Group Inc. I have with me today Alan Tracy, Acting Assistant Secretary, Marketing and Inspection Services. At the conclusion of my statement, we will be pleased to respond to any questions you might have. My remarks are made for the Department as a whole.

The development of USDA's policy on biotechnology is part of a government-wide effort to provide a coordinated framework for regulating this new technology. At this time, I would like to discuss briefly USDA's policy initiatives in this area.

In response to the anticipated growth in the commercial application of genetic engineering technology, the Office of Science and Technology Policy (OSTP) in the Executive Office of the President published a "Proposal for a Coordinated Framework for Regulation of Biotechnology" on December 31, 1984 (49 FR 50856-50907) (hereafter December 84 Notice). The OSTP proposal included policy statements by the Food and Drug Administration (FDA), the Environmental

Protection Agency (EPA), and the U.S. Department of Agriculture (USDA). As a part of its policy perspective, USDA believes that agriculture and forestry products developed by biotechnology will not differ fundamentally from conventionally produced products and that the existing regulatory framework is adequate to regulate biotechnology. USDA also has pledged to amend its regulations if developments indicated a need for additional oversight.

In the months since the OSTP proposal was published, USDA has attempted to encourage promising developments in agricultural biotechnology, in both USDA-funded research projects and commercial product development, while protecting human health and the environment through the administration of existing statutes. We have taken a number of steps to put our biotechnology policy into effect.

First of all, I am pleased to report to this Committee, that the Secretary has approved the establishment of an Office of Agriculture Biotechnology (OAB), which will have primary responsibility for implementing and coordinating the Department's policies and procedures pertaining to all facets of biotechnology. This includes the conduct of laboratory and field research, experimentation on biotechnological products prior to their commercialization, and all matters of oversight of biotechnology in agriculture. The new Office will report to the Assistant Secretary for Science and Education and perform a staff role for the interagency committee on biotechnology in Agriculture which will be known as the Agricultural Biotechnology and Recombinant DNA Advisory Committee (ABRAC), and for a National Biological Impact Assessment Program. The Assistant Secretary for Marketing and Inspection Services will continue to exercise the Department's existing regulatory responsibilities for products developed through research.

Your first question concerned APHIS' development of guidelines for licensing veterinary biologics derived by recombinant DNA-techniques, and the evolution of those guidelines from USDA's Federal Register Notice of December 31, 1984 (49 FR 50897).

In a July 19, 1985, Federal Register Notice (50 FR 29367-68) the Secretary of Agriculture delegated authority for coordinating the development and the carrying out of all matters and functions pertaining to the Department's regulation of biotechnology to the Assistant Secretary for Marketing and Inspection Services. These responsibilities were further delegated to the Administrator of the APHIS. In this same notice the Secretary of Agriculture delegated responsibility for coordinating the development and the carrying out of all matters pertaining to USDA funded agricultural research in biotechnology to the Assistant Secretary for Science and Education.

An understanding of the way in which USDA has divided these responsibilities should prove useful to those in the private sector seeking review and/or approval of biotechnology applications. The Assistant Secretary for Science and Education (S&E) oversees the research activities for the Agricultural Research Service (ARS), the Cooperative State Research Service (CSRS), the Extension Service (ES), and the Office of Grants and Program Systems (OGPS). The Assistant Secretary for Marketing and Inspection Services oversees the regulatory activities of APHIS, which includes Veterinary Services (VS) and Plant Protection and Quarantine (PPQ); the Agricultural Marketing Service (AMS), and the Food Safety and Inspection Service (FSIS). USDA-funded research projects involving genetic engineering are currently overseen by the USDA Agricultural Recombinant DNA Research Committee (ARRC). Applications for veterinary biologics licenses and permits and permit applications for plant pest

movement are regulated by APHIS. In the performance of these research and regulatory activities, there will be close coordination between the research and regulatory agencies in all cases involving genetically engineered organisms.

The Administrator of APHIS established a Biotechnology and Environmental Coordination Staff to assist him in fulfilling his regulatory responsibilities for biotechnology. This staff coordinates biotechnology regulatory initiatives. The staff serves as a liaison between APHIS and other USDA agencies as well as other Federal agencies.

On September 23, 1985, APHIS published guidelines in the Federal Register (50 FR 38561-63) for the control and protection of documents containing confidential business information (CBI) concerning biotechnology. The protection of CBI is understandably a concern of industry, and the protection of trade secrets was urged by numerous commenters representing biotechnology companies. The USDA guidelines are based upon the confidential business information exception contained in the Freedom of Information Act (FOIA).

USDA is preparing a Departmental policy statement on research and regulation of biotechnology for publication in the Federal Register. Similar statements are being prepared by FDA, EPA, NIH, and OSHA as a part of a coordinated policy statement that refines and expands upon the December 1984 Notice. The USDA policy statement also will describe proposed guidelines for oversight and safe conduct of federally funded agricultural biotechnology research.

The USDA policy statement includes a description of proposed new regulations under the authority of the Federal Plant Pest Act (FPPA) and the Plant Quarantine Act (PQA) for genetically engineered organisms which are plant pests. Also explained in the USDA policy statement are the procedures APHIS follows in licensing veterinary biologics regulated under the Virus-Serum-Toxin Act (VSTA) which may be derived by recombinant DNA techniques or developed from hybridomas.

Among these USDA policy initiatives, the procedures for licensing veterinary biological products derived by recombinant DNA techniques pursuant to the authority of the VSTA are the topic of concern to this audience.

Under the Virus-Serum-Toxin Act of 1913, 21 U.S.C. 151-158, the USDA (APHIS) (VS) has exercised regulatory authority over all veterinary biologics imported into the United States or shipped or delivered for shipment interstate. Recent amendments contained in the Food Security Act of 1985 have extended this authority to products which are shipped intrastate or exported, and have given the Department additional enforcement mechanisms such as the power to detain and seize products. Under the VSTA, veterinary biologics may not be shipped or delivered for shipment if they are worthless, contaminated, dangerous, or harmful.

For the purposes of licensing, biologics derived by recombinant DNA-techniques or developed from hybridomas, may be classified into three broad categories based on the biological characteristics of the new products and the safety concerns they present.

The first category includes inactivated recombinant DNA-derived vaccines, bacterins, bacterin-toxoids, virus subunits, or bacterial subunits. These nonviable or killed products pose no risk to the environment and present no new or unusual safety concerns. Monoclonal antibody (hybridoma) products used prophylactically, therapeutically, or as components or diagnostic kits also are included in this category.

The second category includes those products containing live microorganisms that have been modified by the addition or deletion of one or more genes. Deleted genes may code for virulence, oncogenicity, enzyme activity, or other biochemical functions. Added genes may result in the expression of new immunizing antigens or the production of novel biochemical byproducts such as beta-galactosidase. Precautions must be exercised to assure that this addition of specific genetic information does not impart increased virulence, pathogenicity, or survival advantages in these organisms which are greater than those found in natural or wild-type forms. It is important that genes added do not compromise the safety characteristics of the organisms. In most cases it is expected that they will be improved, and would therefore not pose any new threat to humans, other animal species, or to the environment.

The genetic information to be added must consist of well-characterized DNA segments. A comparison is also required to be made between the genetically engineered organism and the wild-type form with respect to biochemical pathways, virulence traits, or other factors affecting pathogenicity.

The third category includes products using live vectors to carry recombinant-derived foreign genes that code for immunizing antigens and/or other immune stimulants. Live vectors may carry multiple recombinant-derived foreign genes since they can carry large quantities of new genetic information. They also are efficient at infecting and immunizing target animal species. These properties, for example, make vaccinia virus recombinants very popular subjects for vaccine development programs. Characteristics of safety and transmission must be answered fully before questions and concerns dealing with safety to humans, animals, and release into the environment can be answered and before products can be considered for licensing.

TechAmerica Groups Inc. was issued a license for a genetically engineered thymidine kinase negative (TK-) pseudorabies vaccine, which is a second category product because it contains live microorganisms that have been modified by the deletion of a gene. The chronology for issuing the license to TechAmerica Group, Inc. has been distributed to this committee. You have also received copies of the Environmental Assessment prepared by APHIS's Veterinary Services in the licensing of the vaccine.

I would like now to discuss the matter of the voluntary suspension of sales of OMNIVAC by TechAmerica Group, Inc. The procedures followed by the Department in granting the license were consistent with USDA regulatory procedures. The voluntary suspension was agreed to by the Department and TechAmerica to permit USDA to provide full documentation describing the analytical steps that had been taken to ensure that the product was environmentally safe. The two-week suspension agreed upon by USDA and TechAmerica, in no way suggested any lack of confidence by the Department or the manufacturer in the safety and efficacy of this product or in the adequacy of the measures USDA took to carry out our responsibilities under the Virus-Serum-Toxin Act.

Environmental questions about stability, survival, persistence, and dissemination were identified and resolved by APHIS officials prior to licensing of the vaccine. The Environmental Assessment, now available, fully documents the Department's review and analysis of environmental impacts and supports the Department's licensing of this product.

You asked additional questions concerning the role of Science and Education and the ARRC in reviewing requests for field testing genetically engineered organisms. USDA is in the process of publishing USDA Guidelines for Biotechnology Research that will closely parallel the NIH Guidelines and will be issued under the authority of the Food Security Act of 1985 (Public Law 99-198). The amendment gave the Secretary of Agriculture responsibility for establishing "appropriate controls with respect to the development and use of the application of biotechnology to agriculture." All federally funded agriculture biotechnology research or agriculture research conducted at an institution receiving USDA funds would be subject to the USDA Guidelines for Biotechnology Research unless the specific research project is supported by and subject to the guidelines or regulations of another Federal agency. These Guidelines would encompass all phases of agricultural biotechnology research such as: contained laboratory experiments; specialized isolation research, for example a greenhouse or a biotron; and research involving release into the environment. To encourage voluntary compliance from industry, USDA proposes to adopt the NIH policy of providing the researchers not required to comply with these Guidelines the opportunity to have their new biotechnology research proposals voluntarily reviewed by USDA.

We have developed a system of oversight of biotechnology research in agriculture based largely on our participation in the NIH-RAC process for the past 10 years through the NIH-RAC. I will propose that the Secretary replace the current Agricultural Recombinant DNA Research Committee (ARRC), with a committee closely patterned after the existing NIH-RAC.

We are in the process of establishing a National Biological Impact Assessment Program to provide support in the evaluation and monitoring of the potential impacts of biotechnological processes and products on safety and the environment.

Now more specifically, let me share the following review of genetically engineered products mentioned above. From the earliest concerns about recombinant-DNA research, and prior to the Asilomar Conference in 1975 to discuss these concerns, USDA has been actively involved. USDA was invited to participate in the Asilomar Conference, and USDA representatives met with National Institutes of Health (NIH) officials during the formative stages of their recombinant-DNA advisory committee generally known as the NIH/RAC. Agricultural scientists have been in attendance and members of the NIH/RAC since its first meeting. Agricultural scientists, including a member of the USDA Agricultural Recombinant DNA-Research Committee (ARRC), are members of the NIH/RAC Plant Working Group.

A major portion of the Agricultural Recombinant DNA Research Committee's (ARRC) time since the fall of 1983 has been devoted to helping to develop the USDA's statement of policy for Research and Regulation of Biotechnology Processes and Products. This statement of policy has been in response to the Office of Science and Technology Policy's effort to provide a coordinated framework for regulation of biotechnology.

ARRC's role in the review of applications to field test genetically-engineered products is to provide scientific advice. In the University of California proposal to field test a genetically engineered or "ice-minus" bacterium, the NIH/RAC recommended approval for of the field test, but only after asking the ARRC to also review this request and make a recommendation to it. The ARRC conducted their own review and recommended to the NIH/RAC that the proposed field test be approved. This is the procedure that has been followed by the NIH/RAC and the ARRC in dealing with any agriculturally-related requests that have come to the NIH/RAC.

Current EPA policy states that they must be notified prior to all small scale field tests involving certain microbial pesticides, in order to determine whether experimental use permits are required. This is a change from their earlier policy that prior notification and experimental use permits were not required for any 10 acre or less field tests of microbial pesticides. Their new policy also emphasizes the inclusion of microorganisms genetically altered or manipulated by humans. With this EPA announced policy and the OSTP's efforts over the past couple of years to develop a better coordinated approach in the regulation of biotechnology, the ARRC has been invited to send representatives

to sit in on several of EPA's scientific advisory panel sessions considering prior notification of desired small scale field testing of genetically engineered microbial pesticides.

The ARRC has reviewed only one proposal for field testing of a genetically-engineered product since publication of the Department's proposed biotechnology regulatory policy in the Federal Register notice of December 31, 1984 and that was a product produced by Calgene Inc. as to which review is not final. The ARRC did not review the "field" testing of OMNIVAC. However, such review is not required by the Department's licensing procedures.

My colleague and I will now be pleased to answer questions.

Mr. VOLKMER. Thank you very much, Dr. Bentley.

Again, I must express—I guess first I would like to say I welcome you here and am pleased to have you, but also express my disappointment in not being able to discuss the matters with Dr. Jordan, Dr. Shipley—Shibley, and Mr. Hawkins. But so be it, perhaps we can work that out so we can still do that in the future.

In light of the testimony that we had, especially from Dr. Jordan, back in December—just last December—I was very curious to try to find out from him, in his testimony it was my understanding he said that USDA would be following the guidelines and ARRC would be reviewing these proposals that are genetically engineered, or recombinant DNA organisms; and I then find out in November, right before that testimony, they had classified one and he—they had never heard of it and never got to review it. So that's—that's—we really were concerned about what's going on.

Now I find in your testimony—and this is what bothers me, Dr. Bentley—that you say the ARRC did not review the field testing of Omnivac. Now, I can understand perhaps some of that was done before the ARRC was ever—

Dr. BENTLEY. Yeah.

Mr. VOLKMER [continuing]. Created. But are you telling me—let's change the timeframes a little bit. Let's assume that the publication of the guidelines had been in December 1982, and during the timeframe of 1983 and 1984, that ARRC had been in existence and that the applications, et cetera—or even let's say, Dr. Kit had requested consideration for the purpose of doing field testing on the Maddox farm. You mean ARRC would not have reviewed that; is that what you are telling me?

Dr. BENTLEY. Well, when you—let me go back to some—for general comments to be sure that I have the premise on which I am going to answer. If it were a field test involving a microorganism, a DNA modified microorganism, or plant, that there would be a process for reviewing of field tests.

In the case that we have before us on the pseudorabies vaccine, that this review is—was done at the IBC level, and that the nature of the products—a virus—which is nonreplicating, and so forth—that was not required. A review by ARRC was not—would not be required as I understand what the current regulations are dealing with the Virus Serum-Toxin Act—and I turn to my colleague here to verify that it is not required because of the nature of the—of the product. However, if in looking at the review it could well be—it would be supportive if it were requested and could be helpful to the people concerned with the licensing product. It could be—it could be reviewed and it would be—and we would—then the ARRC would be available.

Mr. VOLKMER. Now, now I'm a little bit more confused. And I'd hate to be somebody out there in industry in this biotechnical—biotechnology field and try and determine what you all are going to do.

Are you telling me—and I—my impression of—Dr. Kit felt that as long as this viral matter—product—that as such, and if you are just removing a gene, that it is not then what you would consider a product that would have to go before the ARRC? Is that what you are telling me?

Dr. BENTLEY. Let me see if I can clarify.

Mr. VOLKMER. Even though it's recombinant DNA?

Dr. BENTLEY. Well, this is still a question, right.

Let's start out with a case of a—where it's a clear-cut recombinant DNA and a new type of an organism or a modification in a plant where you need field—and field testing is a part of the normal research. I am speaking now on the research side. That would be subject to review and the issuance of—of approval under the ARRC process just as it is under the NIH/RAC.

Mr. VOLKMER. Now—now we are talking about a product in which you have a new gene that's been—

Dr. BENTLEY. You might synthesize the gene or was a clearly recombinant DNA or a mod—new type of an organism, and that would be clearly a case that would require approval. Now—and would be—then further, that this was a federally funded research, was not in the private—was not a private concern. Now, there's a question—there's not an absolute obligation, as I understand our ruling, that a company, for example, wouldn't have to. But we are finding most companies want to do this. This is the impression I have.

Now, that would be one set of circumstances. Now, if you come up through another set of circumstances wherein this is a gene deletion, when we talk about the pseudorabies gets specific, and the pseudorabies, there is a question whether or not that needed to go before the RAC. In fact, the current regulations are that they did not have to go before the RAC provided there had been review at the IBC level. And that was deemed to have been done in this case.

I guess there is the additional statement depending upon whether you are talking about a recombinant DNA-derived product such as was the case of the vaccine that we are talking about today.

Mr. TRACY. Mr. Chairman, may I comment?

Mr. VOLKMER. Yes.

Mr. TRACY. I think it is important to differentiate between review under the NIH guidelines for research, which clearly was done in this case, and would be done in the future, and application for licensing under a specific set of regulations. In this case there is a body of regulation developed under formal rulemaking that stems from the Virus-Serum-Toxin Act—Virus-Serum-Toxin Act, and those regulations set out what is required for licensing. Under those regulations there is no requirement that the products be re-submitted to the RAC, or to that process.

We do expect that products which would come from recombinant type research would have been—the research on those products would have taken place under the guidelines prior to its having been submitted for licensing under the VST Act, and that was the case with regard to this virus.

Mr. VOLKMER. Well, let me ask you this: Now, your—you say you had assumed. Wouldn't you require proof that they had followed those guidelines?

Mr. TRACY. Well, we have all the backup data that showed that it had gone through several IBC's, including having gone through our own National Vet Services Laboratory in Ames prior to submission of the license. So it was very clear when we received the

application that it had taken place under IBC review and, therefore—

Mr. VOLKMER. There have been no IBC review on any field tests?

Mr. TRACY. Pardon?

Mr. VOLKMER. There have been no IBC review on any field tests?

Mr. TRACY. Well—

Mr. VOLKMER. Been no IBC approval?

Mr. TRACY. Pardon?

Mr. VOLKMER. The only laboratory tests were all that had taken place? Field tests were not reviewed by anybody?

Mr. TRACY. Well, I don't know that that's correct.

Mr. VOLKMER. Well, let's assume that. Then what would be your position?

Mr. TRACY. Well, I—go ahead.

Dr. BENTLEY. I think Dr. Kit answered that in part. In other words, if there's a field test that involves a, say, a university, there would be—and a set of guidelines for that university, and they would be subject to that control.

Mr. VOLKMER. Well, I—I think we are missing—at least you are missing my point, my concern.

Dr. BENTLEY. Yeah.

Mr. VOLKMER. My concern is this: Let's assume that you have research—and I don't care if the research is funded by NIH, USDA, or who, private, and the research is funded. And the research is done on a matter, whether it is recombinant DNA under your third category or second category, either one.

Dr. BENTLEY. Yeah.

Mr. VOLKMER. And let's say that whoever has—whether it's industry or—or university—they have an IBC and they follow it. And they get approval before they do the lab tests, and it's all done—and done that way. However, then they go out on their own and do field tests without any approval from anybody. And then they come in and ask for an application—make application to license it, and sell it.

Mr. Tracy, from my understanding of what you have told us just a minute ago, it doesn't make any difference—

Mr. TRACY. Well, we don't—

Mr. VOLKMER [continuing]. Whether the field tests were done under an approval or disapproval, or no approval.

Mr. TRACY. Well, for the purposes of licensing under the act, we in effect start the procedure all over. We do our own safety review. We do our own review of potency and efficacy, and of purity. And as you recall, in this particular case, that process took over a year. It was a rather extensive process—which is laid out in considerable detail in the environmental assessment, which Dr. Bentley has asked be included in the record.

Mr. VOLKMER. We can include that in the record without any problem. But here's my problem that, are you telling people to go out here and just go ahead and do anything they want to do and test any way they want to test without anybody's approval, and it won't make any difference?

That's what I'm hearing.

Mr. TRACY. No; of course not.

Dr. BENTLEY. No.

Any—let me—if I can answer. Any product that's done—been genetically modified has—would come under these review and there would be a suitable protocol presented and approved. It would first be looked at at the IBC. If they felt they could answer the questions based upon the regulations and responsibilities that can be handled at the IBC level, they would do so, and there would be a notification—I think the new guidelines will suggest notification. If such one came forward and I were in agriculture, it would be reviewed by our Biotechnology Review Committee and a protocol would approve, with conditions, would be notified to the person doing the field—or doing the testing in the environment.

That is—that is the way it has been operating, and that is the way it would operate in the future.

Now, on the virus Serum-Toxin Act, there's a set of regulations with regard to licensing that Mr. Tracy has talked about. But any testing in the environment of such a product would be subject to review.

Mr. TRACY. May I add that under our current regulations under the VST Act, any intra or interstate shipment of virus for field testing procedures requires a permission from Veterinary Services. We have not had, up until now, the authority to require that for intrastate.

Mr. VOLKMER. You now have it through the 1985 act.

Mr. TRACY. We do have it through the 1985 Farm Act and expect to have implementing regulations published soon.

Mr. VOLKMER. Yeah, but what will that mean, it just says they notify you, and you say Ok, go ahead?

Mr. TRACY. No, sir; there is a detailed process that must be followed listing a number of specific requirements in order for permission to be granted.

In fact, I think we have that list.

Mr. VOLKMER. Dr. Bentley, did you see the article in the New York Times on April 8, 1986, in regard to this?

Dr. BENTLEY. I have seen several articles. I don't know what that particular one is.

Mr. VOLKMER. This is reported by Keith Schneider, dated Washington, April 7, and the second paragraph, it says:

The official, Orville G. Bentley, Assistant Secretary for Science and Education, said the application to test and market a genetically engineered biovaccine should have been brought before the Department's Recombinant DNA Research Committee, a body of experts established 8 years ago to review research and field test for living gene altered products. The agency, quote, "could have asked, and should have asked, the scientists on that committee to give their opinion," said Dr. Bentley.

Is that a proper quote?

Dr. BENTLEY. Reasonably—partly, yes.

The statement there is now in the context of the review within the Department. The Department there—there is—with the rules and regulations relative to licensing of this product does not require that they go before the ARRC committee, or whatever, to the successor organization what that would be—because they felt—it was their judgment that it had been reviewed at—by a peer group at the IBC level.

My context for making that was one in retrospect, and that's the part that's not put in. Perhaps it would have been useful, and sup-

portive, and that's why I speak of the ARRC as being supportive. It doesn't make decisions. It makes recommendations, just like the NIH/RAC makes recommendations to Dr. Wyngaarden as to what action will be taken. My comment was made in—in the context as saying it could have been helpful to have answered some questions that we put—put before the committee that might have been raised by members of the committee—undoubtedly the answer—I don't know what the answer would be. But it would be my assumption that the answer would have been the same as it—as it is now, that there wasn't any need for further review.

Mr. BEDELL. Will the gentleman yield?

Mr. VOLKMER. Yes.

Mr. BEDELL. It doesn't say could. It says could have asked, and should have asked, if it's a statement, Dr. Bentley.

Dr. BENTLEY. I can't say, Mr. Bedell, whether I said should or not. I can't answer to that question whether I said it should be. I'd—I had talked to him about in retrospect it would have—could have been useful and helpful.

Mr. BEDELL. Will the gentleman yield further?

Mr. VOLKMER. Yes, go ahead.

Mr. BEDELL. It should have been?

Dr. BENTLEY. My—what I say—

Mr. BEDELL. Now.

Dr. BENTLEY. Yeah, in retrospect perhaps it would have been helpful if we would have done so. But that doesn't necessarily mean that it would have been the outcome would be any different.

Mr. BEDELL. But you do agree that it should have been?

Dr. BENTLEY. Should—it should have been—it could have been—been helpful. Maybe you answered a lot of questions that have come up. But I don't think it would have changed it.

Mr. BEDELL. Will the gentleman yield further?

Mr. VOLKMER. Yes.

Mr. BEDELL. Let me—let me finish this up.

Dr. Bentley, what you are telling me is that you still don't have it straight over there at USDA what must be done. That's—that's what some of us are looking for, a clear road for people to follow and that we know that people are following. When you say, well, it should have been done but it really wasn't necessary to be done, and you can do it if you want to, it might clear things up afterward and answer some questions, but you really don't have to do it. And what you are telling people out there by that saying that, I am afraid is, that as long as it's a viral genetically altered gene, by only taking away a gene instead of putting anything in it, you just go ahead and do what you want to do out there.

Dr. BENTLEY. Mr. Chairman, I would like to state this. First, there is no condition that I know of that there is need for review by a peer group. Now, and if there is, I—I will be—stand corrected.

Mr. TRACY. For licensing.

Dr. BENTLEY. For licensing.

But even in the licensing case, there was review at the IBC level by a peer group.

Mr. VOLKMER. Who? Who?

Dr. BENTLEY. Baylor.

Mr. VOLKMER. No. I disagree. Only for lab.

Dr. BENTLEY. We are talking about the field test. I am talking about—about the—it was done by—in Texas at the Maddox location was—

Mr. VOLKMER. No IBC?

Dr. BENTLEY. No IBC, but it was under a quarantine situation and under the direction of the Commissioner whether it is animal science—or livestock, or health, in Texas, so that was a question.

But I won't necessarily pass on that particular question whether or not it was a misinterpretation. You have the question there about using a nonreplicating virus by one of the most able scientists in America, and—and by people that are responsible for understanding and handling vaccines. But we must remember that we are veterinarians and there's a question of whether or not an animal is a contained unit. That issue has to be taken—the scientific evidence that there's no shedding; that it is not transmissible; it's not a replicating organism because it's a virus, a chemical, in a sense, not an organ—living organism. It had been derived from a gene—you've got all of these questions that make a difference in this particular case.

Mr. VOLKMER. Dr. Bentley, I'm afraid that—I'm just getting very upset by what you just said. What you just said to a layman is that we know all about these things, about this virus, and we know all these things about any genetic altered mutation of this virus, and we know it's safe, and, therefore, we really don't have to worry about it.

That was the same attitude that AGS took before this subcommittee and before, when they are out on a roof out in California and started pumping stuff in the trees—and the same attitude.

I—I'm afraid that the attitude of my—at least myself, and I think many of my public that I have talked to around Missouri on this, is that the attitude is nothing is safe. We've started out with nothing is safe and that you go through the procedure it's safe. How do you know before it's tested that it didn't shed?

How did you know before it's tested that it doesn't shed? Tell me.

Dr. BENTLEY. Laboratory. That's—this was the purpose of the work that was done in laboratories, and where it was under—and they were in full compliance with the rules because they were in restricted facilities—the one in Ames Lab and Michigan State Center, the work that was done at the—at Baylor, and the laboratories. All of those were under contained facilities—P-2 or P-3 level—and they did those kinds of tests at that time, yes.

So, I agree with you, you have to—

Mr. VOLKMER. Those laboratory tests were done. OK.

The investigators were satisfied that these things took place, whatever they were.

Dr. BENTLEY. Yes.

Mr. VOLKMER. And then basically all that—

Dr. BENTLEY. That is my understanding, yes.

Mr. VOLKMER. Basically, all positive.

Now, we say, OK, since it is done in the laboratory, then you can go out and go ahead and do the field testing without anybody reviewing what you have done.

Now, some of us feel that somebody else ought to be reviewing that to make sure. That's all—

Dr. BENTLEY. So do I, so do I.

Mr. VOLKMER. No, you just told us they didn't have to. You just told us they didn't have to.

Mr. TRACY. Mr. Chairman, we did review in APHIS all of the field tests that took place after the time of licensing—license application, excuse me.

Mr. VOLKMER. After?

Mr. TRACY. After the time of licensing application.

Mr. VOLKMER. What about field tests that take place before?

Mr. TRACY. Those which were taken place intrastate, we had at that time no authority to require—for a requirement for permission. We now have that authority and we expect to take advantage of that authority for development of new regulations. But we have a very strong body of regulation in place.

Mr. VOLKMER. It's amusing to me that EPA tells AGS that they can't do what they tried to do in California—and that's all in California. But you tell me you can't do anything and it's all in Texas where the matter is taking place—until we pass a law saying you can.

Mr. TRACY. Saying that USDA had no jurisdiction at—under the Virus-Serum-Toxin Act, for intrastate movement of that virus at that time. That has since been corrected by Congress and will be finalized through issuance of formal regulations.

Mr. BEDELL. Will the gentleman yield?

Mr. VOLKMER. Yes, I yield, I am going to quit. I yield to the gentleman from Iowa.

Mr. BEDELL. I don't understand why you need any authority. You are going to license them, depending upon whether or not you feel those tests are satisfactorily carried out or not, aren't they? So I don't understand what authority you need. I don't understand why you need any authority. You are going to license them. And you license them based upon your satisfaction of the tests were satisfactory. And if they were not satisfactory, you don't need any authority, you just don't license them. I don't understand.

Mr. TRACY. No, this is for tests that take place before the license is issued.

Mr. BEDELL. Right.

Mr. TRACY. And we do have to give permission for that. Currently, under any intra—

Mr. BEDELL. No, no.

Mr. TRACY [continuing]. Or interstate movement of the product.

Mr. BEDELL. I understand. But you did have—you license just not just in Texas, you licensed for across the country, did you not?

Mr. TRACY. Yes.

Mr. BEDELL. You had that complete authority as to whether or not you would license it across the country, did you not?

Mr. TRACY. That's correct.

Mr. BEDELL. You didn't need legislation to determine that.

Mr. TRACY. That's correct.

Mr. BEDELL. And wasn't one of the requirements that you should have looked at as to whether tests were properly conducted, and so on?

Mr. TRACY. Yes, we looked at the tests that were conducted. The body of regulation is there to assist us in determining whether the

product is safe; whether it's potent, pure, and—and effective. And we looked at all of the tests that have taken place. We have no authority under that act for those tests prior to the license application.

Mr. BEDELL. I understand all that. But if the tests are improperly conducted, and so on, you certainly have the authority to not license it if you wish—if you feel it should not be licensed.

Mr. TRACY. We have no basis to say that any tests were improperly conducted.

Mr. BEDELL. And you don't think it's up to you to look at that, and determine that?

Mr. TRACY. Well—

Mr. BEDELL. You see, the worry we've got is this isn't this issue. The worry we have is in the future, if we release things that shouldn't be released, and apparently you don't think that's your worry.

Mr. TRACY. No, we're talking about two separate items here. The tests were part of the research which was taking place under guidelines. The—

Mr. BEDELL. Wait a minute. Under supposed guide—supposedly taking place under guidelines.

Mr. TRACY. That's right. And we received the license application—we, in APHIS, essentially have no role. We review the material. We review all material that's made available to us and, of course, go back and request considerable material to determine whether the product is indeed safe. And all tests that have taken place before, no matter who approved them, would be things that we would look at and try to determine whether the product is safe.

Mr. BEDELL. But the act requires, does it not, compliance with NIH guidelines? Is that right?

Mr. TRACY. The act in and of itself does not require compliance with NIH guidelines. We do have a policy statement that was published in the Federal Register in 1984—December 31—which says that USDA requires all license applicants for products derived from DNA technology to comply with NIH guidelines for research involving recombinant DNA molecules.

The very same piece of—the very same document also says that veterinary biological products prepared using modern biotechnological procedures such as recombinant DNA, chemical synthesis, or hybridoma technology will be treated similarly to products prepared by conventional techniques. And each product is evaluated individually to determine what will be necessary to establish its purity, safety, potency, and efficacy. That is what we do under the—

Mr. BEDELL. But your regulations require compliance with NIH guidelines. Is that correct or incorrect?

Mr. TRACY. No.

Mr. BEDELL. Oh, they don't?

Mr. TRACY. No. We have a policy statement—

Mr. BEDELL. You don't care whether they comply with NIH guidelines or not—

Mr. TRACY. The regulations deal with licensing.

Mr. BEDELL. I understand.

Mr. TRACY. The NIH guidelines deal with research.

Mr. BEDELL. And your licensing procedure goes forward regardless of whether the research has been properly conducted under NIH guidelines or not; is that correct?

Mr. TRACY. We do require that in products dealing with—involving recombinant DNA molecules that the research was done under NIH guidelines.

Mr. BEDELL. You do require that?

Mr. TRACY. Yes.

Mr. BEDELL. Well, if you do require that, how do you—

Mr. TRACY. We do that through policy rather than through a formal statement in—

Mr. BEDELL. But if you require it—if you require somebody to do something, then you must have to satisfy yourself that they have done that. How do you satisfy yourself that that's been done?

You can't require something unless satisfying yourself that it has been done.

Mr. TRACY. Like we said, we have no basis for saying that any of the research that was done in this case was not done under NIH guidelines—

Mr. BEDELL. That's not the issue. Mr. Tracy—Dr. Tracy—

Mr. TRACY [continuing]. Without having a considerable body of information which says that there was—that the—this product was reviewed under NIH guidelines, including several IBC reviews.

Mr. BEDELL. This isn't a question of whether you can prove it wrong or not.

The question is, If you require that, then it seems to me that you have a burden to see that it has been done according to your requirements. Do you disagree with that? You don't think you do?

Mr. TRACY [continuing]. As a matter of policy, we do not have anything in the VST regulation which says that we must—

Mr. BEDELL. Forget all this jargon.

If you require—if you require that they comply with NIH Guidelines, which apparently you say you do—

Mr. TRACY. I'm sorry. I, perhaps, spoke too strongly by using the word "required" then. We—

Mr. BEDELL. You read it. Isn't that what it said?

Mr. TRACY. Well, that is, again, a proposed policy statement. That is not a regulation.

Mr. BEDELL. Well, then your policy is that you don't care whether they follow the NIH guidelines; is that correct?

Mr. TRACY. No, that is not correct.

Mr. BEDELL. Well, one or the other. Either you require it or you don't require it. Do you require it or don't you require it?

Mr. TRACY. We expect them to do it and we do not have any formalized review procedure to ensure that they have.

We do, however, have plenty of opportunity to see what was done. And it is pretty apparent when you look at the tremendous body of—of work done here that this was reviewed under NIH guidelines.

Mr. BEDELL. So—so you do not require it?—apparently. You would like to have it done but you don't require it?

Mr. TRACY. I will correct my original earlier statement and agree with you, sir.

Mr. BEDELL. OK. Because if that's the case, I think this is a terrible deficiency, then, in your operation. If you don't, you know, go ahead and get a license even though you haven't followed the guidelines that you think ought to be followed. But that's beside the point.

Go ahead, Dr. Bentley.

Dr. BENTLEY. I'd like to comment on the—on the fact that when you—we'll talk now on the research. We are coming up with a—we'll take someone like in this kind of a situation, they worked in the greenhouse and they have a plant that's been modified, or some gene introduced into it and so on—that if that proposal came through the IBC process, we would anticipate it would go through that, that it would go through the review process in the Department, and it would be approved. And then if approved, there would be a recommendation made for approval by that—they would establish a protocol for what to be done for testing, say a quarter of an acre, or a small plot, or whatever it was, to test this as a plant, and plants.

Then that would be approved and there would have to be a reporting back—it's assumed that there would be a obey—that people would comply with that—most—and we would anticipate that people would comply with that requirement for testing in the environment.

If not, then I—they—whether or not the penalties—I'm not sure as I wouldn't—can recall from memory—penalties that might be associated with the NIH guidelines or the USDA guidelines. But in the case of EPA, there are penalties for—for noncompliance, and that would be—would have to be a matter of enforcement to comply. Yes, we would want those kind of reviews and we would expect that people will do this. I would expect that even in the case of industry where there might not be an absolute authority that they would request that many industries in the biotechnology, I would anticipate would ask—put their proposals forward. We would review them—and we would agree or disagree. And if there was agreement, they set protocols for doing this. And this is the way we hope to operate.

Mr. BEDELL. So you assume and hope that they would do that and expect they would do it—

Dr. BENTLEY. Yes, I would think if they would—

Mr. BEDELL. Let me finish, please.

Dr. BENTLEY. Oh, excuse me, I'm sorry.

Mr. BEDELL. I would appreciate it if you didn't interrupt me, Dr. Bentley.

You assume and hope that they will do it, but you do not require that they do it?

Dr. BENTLEY. Yes, we would require.

Mr. BEDELL. Well, Mr. Tracy just testified you didn't require it.

Dr. BENTLEY. This is in the licensing. I'm talking about the research level of this. And the licensing of the process—of the product now, and we will get to the product. It should have gone through the review process—in fact, the research, if it was a research process—and that in the product there would be review at the IBC level, and there would be—

Mr. BEDELL. But you don't check to see whether it was done or not? Reviewed at the IBC level.

Dr. BENTLEY. Yes, unless I am wrong, I think we would check, and we would know—a part of the documentation.

Mr. BEDELL. Would you require it? Is that part of your policy that—is that part of the policy?

Dr. BENTLEY. As a part of the documentation that would be submitted for licensing that the person submitting the license would document what they've done.

Mr. BEDELL. Well, this is sure confusing. You would like to have them do it, but you don't require it, as I understand it. And you don't require any of these things, but you would like to have them do it, and would expect that they would. I guess some of us would feel that that's not a very good policy. We sort of—but that's up to you as to how you run your department. I think Congress has some oversight, though. But if that's the way it is, why, that's what we want to know.

I yield back the balance of my time.

Mr. VOLKMER. I recognize the gentleman from California.

Mr. PACKARD. I think much of what I wanted to pursue has already been discussed.

Let me simply ask, Dr. Bentley, were any of the officials at—at the Science and Education branch of USDA, including ARRC, were they aware of Dr. Kit's field test of Omnivac in June 1984?

Dr. BENTLEY. I, from my own personal knowledge, can't answer that for sure.

Mr. PACKARD. To your knowledge, no one at—

Dr. BENTLEY. Well, I think they may have known about it because of other connections. But I can't answer whether or not they did, myself. I would turn to—

Mr. PACKARD. Dr. Tracy?

Dr. BENTLEY [continuing]. Others.

Mr. TRACY. I am unaware of either. Our process basically starts with receiving the license application—and now it's before a license.

Mr. PACKARD. So, are we then to assume if no officials were aware of it, that the field tests were not NIH approved?

Dr. BENTLEY. That is my understanding, that the approval—what approval process had to do with those developed at—in Texas, relative to where the test was conducted by trained people under this auspices of—

Mr. PACKARD. And then did I hear correctly, and am I correct in assuming also, that—that intrastate does not require NIH approval of field testing?

Mr. TRACY. Excuse me—the difference between intrastate and interstate is under USDA—under the—excuse me—under VST, Virus-Serum-Toxin Act, licensing. We—if the product is in the licensing process we can allow tests to go forward inter—interstate, with approval, following a rather substantial series of requirements. We have not, until now, had the authority to require that inter—intrastate.

Mr. PACKARD. But that does not—does not then, you are saying, does not include field testing?

Mr. TRACY. Your question with regard to NIH guidelines, my comment is that the intrastate—interstate difference only is relevant with regard to exemption to be granted for field testing under, and movement of product, to be granted by Vet Services under the licensing procedure.

Dr. BENTLEY. If I may add, Mr.—

Mr. PACKARD. Dr. Bentley?

Dr. BENTLEY. For any testing—field testing on—of a—utilization of a biotechnology-modified product, that would be interstate; that would be uniform as far as requirements for testing, moving, or approval to test in the environment would have nothing to do with interstate, or State—it would be a hearsay requirement, nationally, going back to President Ford's—

Mr. PACKARD. Did Omnivac fall into that category where—that requirement?

Dr. BENTLEY. Yes, it would fall into the requirement. And that was satisfied, we hope with—it was satisfied through the IBC. But the licensing process is—then it gets to another level.

Mr. PACKARD. But what is subject to ARRC approval and ARRC review? What normally do you understand ARRC is to—

Dr. BENTLEY. The major component that would be reviewed by ARRC would be research projects and protocols having to do with research that would come to the—them as a result of federally funded research as their—as the definition.

It is my feeling that there would be—there will be research requests dealing with funding of—at institutions regardless of source if it fell under the review of the Department of Agriculture, and by many industry people that may or may not be required to do it, they would still be—would still ask—could ask for approval for review.

Mr. PACKARD. Does that review only involve research in the laboratory, or does it involve the field testing of that research?

Dr. BENTLEY. It would include all—it would include the—I think for the question when it would come up, sir, would be when they go for field testing of a product, that is, if there are by guidelines they can tell what they can do in contained facilities, whether there's P-1, P-2 level facilities—they would know that—would be, when they would really ask the question, I wouldn't anticipate. Where the real question would come up is when they are talking about testing in the environment, going outside of contained facilities.

There may be questions even within contained facilities, but I think when we will see the most of it will be when we talk about going into the environment.

Mr. PACKARD. And do you believe that Omnivac falls under the—that—those—the category you have just described as being the review process of ARRC?

Dr. BENTLEY. Well, there's two—there are two levels to it. One question would be whether at—when at the research level, whether or not the provisions under the RAC that says that are—that following the process of the protocol that was used does constitute compliance with the RAC, that should be reviewed. And so now the next part of the question would be that with the ARRC that we are reviewing here. Would it been—could it have been reviewed and

looked at in the Department by the ARRC as a support to the licensing process under the Serum-Toxin Act?

Mr. PACKARD. How often does a product—now we are talking about an actual product.

Dr. BENTLEY. Yes.

Mr. PACKARD. How often does a product that's looking to be licensed come and go to—to ARRC for its review before approval of licensing?

Dr. BENTLEY. We have really only had one case of the type—the pseudorabies case. I believe I am speaking correctly that there's been one case that's been before us, and that was handled, as we've said before, in a certain way. We have had one genetically modified plant that's been through the ARRC review process and has been—followed the same procedure that I have said—we have only two products of the type to—on which we use the basis for experience in agriculture.

Mr. PACKARD. Were those two products handled identically the same in the way that they moved through the review process?

Dr. BENTLEY. Well, one was a research product with the type I described—the Calgene request, is a research—is a request for testing of a modified plant in the environment. That was handled in the way of being looked at by the ARRC and reviewed for—for the EA, and so on. That was one process.

The other process—the other product—has to do with the licensing of the pseudorabies vaccine which is a—at the product level, and would be handled under regulations as established by law and by rule, and in accordance that Mr. Tracy is going to outline. And here is a case where there's a different—could be a difference of opinion, would it have been useful if an ARRC review had been made? The answer is a matter of judgment.

Mr. PACKARD. Mr. Tracy—

Dr. BENTLEY. But in every case it's safety.

Mr. PACKARD. Mr. Tracy, why was the license suspended for two weeks?

Mr. TRACY. First, I should probably correct something. I am not a doctor. But the license was suspended in order to allow us to put the environmental assessment that had been done in proper form to present to the court.

Mr. PACKARD. And what was the outcome of that review?

Mr. TRACY. That review—there's no really review. We had already done all of the safety testing on the product before the original release and we had that well documented but it was not in one—one package in a formalized environmental assessment which could be presented to the court. And so we did that with the concurrence of the company, we temporarily suspended the license so that that could be completed.

It was the environmental assessment and the finding of no significant effect.

Mr. PACKARD. And did that change the granting of the license?

Mr. TRACY. No.

Mr. PACKARD. There was no change in the granting of the license?

Mr. TRACY. No, there was no change. There was no re-review. In fact, it was simply compiling into a slightly different form the information that was already there.

Mr. PACKARD. There is a requirement in, from what I understand, that an EIS is submitted before licensing. Is that correct?

Dr. BENTLEY. Or an assessment.

Mr. PACKARD. Or an assessment, right. Or an assessment which normally would declare that there is no environmental impacts.

Was that done?

Dr. BENTLEY. Yes.

Mr. TRACY. Yes.

Mr. PACKARD. Then why was there a need for—for an assessment later—well, let me ask the question one more time.

Was an EIS provided and published on this particular application?

Mr. TRACY. An EIS, no.

Mr. PACKARD. There was no EIS?

Mr. TRACY. Correct.

Mr. PACKARD. What was done in its place?

Mr. TRACY. Well, in the first place the safety review is the very first part of the licensing procedure and continued on through the entire year plus of licensing. We did the environmental assessment prior to releasing the original license. We did not prepare the environmental assessment piece of paper which is here and presented to you until afterward.

Mr. PACKARD. So you did the environmental assessment previous to licensing?

Mr. TRACY. That's correct. We did all of the safety—

Mr. PACKARD. But the document—

Mr. TRACY [continuing]. The entire safety review which included assessment of any impact on the environment. Yes, all of that safety assessment was done.

Mr. PACKARD. Was that documentation also done? Was it documented?

Mr. TRACY. Yes, it was well documented, and is well documented. It simply was not in the form that we wanted to have it available to present to the court.

Mr. PACKARD. Why wasn't it? Was there an urgency—a time urgency to approve the license before the documentation was completed?

Mr. TRACY. No, it was really done according to our normal procedures.

Mr. PACKARD. Is that the way you normally would do it?

Mr. TRACY. Yes, it is.

Mr. PACKARD. In other words, the licensing could be granted under—under your guidelines before the publishing or the releasing of your environmental assessment?

Mr. TRACY. We are not required to publish or release that environmental assessment.

Mr. PACKARD. I see.

Mr. TRACY. We are required to do an environmental assessment; in other words, an assessment of the safety risk, and that was indeed done.

Mr. PACKARD. Was the assessment the outcome of that assessment—was there a determination that there was no—that it was a negative environmental impact?

Mr. TRACY. It was determined that it was a very safe product.

Mr. PACKARD. OK.

Mr. TRACY. We require—

Mr. PACKARD. So the 2-week—the 2-week suspension was not to evaluate the licensing and publicly make known the assessment?

Mr. TRACY. It was not to evaluate the licensing, no. It was simply to prepare—properly prepare—those documents.

Mr. PACKARD. And that procedure is according to your guidelines?

Mr. TRACY. We had done the assessment—

Mr. PACKARD. Yes?

Mr. TRACY [continuing]. Prior to licensing. I hope I am answering your question.

Mr. PACKARD. On November—let me bring up another point. On November 7—I mean February 7, I'm sorry—two additional purity and identity tests were completed, and OMNIVAC was licensed on January 16. Now, how could the license be granted without all the test results? What was the purpose of the additional test and why—how do you explain the fact that the licensing took place previous to the completion of these tests? Either the tests weren't necessary or they—

Mr. TRACY. What tests are you referring to, the dates?

Mr. PACKARD. Well, the purity and identity tests.

Mr. TRACY. And you're—I'm sorry, I don't have that data here. November what?

Mr. PACKARD. Apparently, from what I understand, those were done by the National Veterinarian—Veterinary Services.

Mr. TRACY. The National Vet Services Laboratory did a number of different things. It did tests on the master seed virus that showed on October 10 that it was satisfactory. In the environmental assessment, which we can give you, on page 4, it goes through the licensing procedure, and we also can provide for the record, if you wish, a chronology of the various steps taken in the license application and in the additional testing.

Mr. PACKARD. Let me ask a concluding question, then, very generic.

Were any tests performed after, to your knowledge, any required tests performed after the licensing was granted?

Mr. TRACY. No.

Mr. PACKARD. That's all.

Mr. VOLKMER. The gentleman from Washington.

Mr. MORRISON. Thank you, Mr. Chairman.

Dr. Bentley and Mr. Tracy, is it fair to assume that part of the confusion that seems to be coming from this hearing is the fact that we are talking about—both about research and licensing procedures at the same time?

Dr. BENTLEY. Yes.

Mr. TRACY. That's very true.

Mr. MORRISON. And that you just can't talk about them both in the same breath and have it make any sense at all?

Mr. TRACY. That's correct.

Mr. MORRISON. OK, thank you.

I guess my major concern, and the only question I will really ask is as I look at what's happened revolving around the case we are investigating today and the response of the agency to it, without making determinations on whether you are guilty or not of jumping from one foot to the other, is it fair to assume that with your new office within USDA—the Office of Agriculture Biotechnology—that new authority that was given you in the 1985 farm bill, new regulations you have pending, that in fact you have your act together now?

Dr. Bentley.

Dr. BENTLEY. The answer to that is "Yes." And that we are in the process—that this is the time when we are in the process of developing a set of guidelines and working with the other agencies of the Federal Government and there's a lot of hard work done by lots of people trying to find answers so that we can put the—all of this in a sort of a coordinated framework. This is very helpful. And the need that—and that will then clarify various steps within each of our agencies.

There is a—two or three guiding principles, though. Certainly we are concerned—deeply concerned about safety, and reducing risk with association with this product or any product that's being licensed, or any process in research. Likewise, we—the stress on peer review, and the value—and depending upon scientific evaluation and in making decisions concerning all matters related to biotechnology.

And then finally, that the process that we have gained in experience—and I think that with the new arrangements that we have, Mr. Congressman, we can answer these questions, and will. We've got a great deal of expertise and knowledge, that we can draw on to find good answers to these questions—to processes, and establish processes that will do—and fulfill our responsibility, and at the same time, help the industry of biotechnology to develop.

Mr. MORRISON. Just one additional question along these same lines.

Part of the driving force that you described in your testimony for getting all these entities of the executive branch together as it concerns the whole arena of biotechnology, you mentioned the Office of Science and Technology. And just wondering if as a result of these hearings if you need a push on other agencies to complete their coordination with your efforts in this whole arena, or do you see all of this coming together? Is the work within the Environmental Protection Agency, for instance, satisfactory in your eyes?

Dr. BENTLEY. I am very pleased to report that it is coming together, and that the leadership of OSTP has been very important. The Biotechnology Working Group that's been meeting almost 2 years now in dealing with these questions, and the Domestic Policy Council that's looking at these overall proposals we have.

I think we've—I think we're in a very strong position at this time. And I look forward to the publication of these guidelines to indicate proof of what I have just said.

Mr. MORRISON. Mr. Tracy, any addition to that?

Mr. TRACY. Just that that publication is likely to take place very soon, hopefully within the next few weeks. And concurrently with

the publication of that broad policy statement just as Dr. Bentley, in his testimony, mentioned, includes a specific policy statement for Agriculture. We hope also to have some regulatory revisions prepared for introduction at about the same time.

Mr. MORRISON. I thank you very much.

Thank you, Mr. Chairman.

Mr. BEDELL. Mr. Chairman?

Mr. VOLKMER. Yes, I recognize the gentleman from Iowa.

Mr. BEDELL. If—if a private company does all the research on a product and then sells it to another company, and that second company applies for a license, do you have—in any way do you hold the first company responsible if they violated NIH regulations in the terms of the way they perform their research?

Mr. TRACY. Maybe I can respond. We are dealing again with licensing. And what we require under licensing is safety of the product.

Mr. BEDELL. So you do not—

Mr. TRACY. What we require is safety, and that safety does have to include peer review. And whether that's through NIH or through internal review systems is reviewed on a case-by-case basis.

Mr. BEDELL. But—but if the peer review wasn't even carried out, then you do not have regulations that in any way affect your licensing of that second company, I take it from your testimony?

Mr. TRACY. If there was no peer review, I think we would have a hard time accepting the data that was presented to us.

Mr. BEDELL. Well, if—if if I developed a genetically derived product and then I went out and tested that without any peer review or anything, and then it was tested and the test said it's all right. And then I sold it to another company, and then that company applied with this documentation that it had been said it was all right, would there be a reason for you to not license this?

Mr. TRACY. We would have to review that documentation to see whether we agreed that it was all right and—

Mr. BEDELL. And the documentation said it was all right even though it was not carried out under NIH guidelines and even though NIH would not have approved putting that out for testing into the public environment, would you—

Mr. TRACY. We start the licensing process. We in effect start fresh when we get—when we get that—that application.

Mr. BEDELL. I understand.

So—so I'm the—I'm the second company and I have bought this from somebody else that did all the research. And as they did the research they didn't pay attention to NIH guidelines or anything, they just went right ahead and did the research. And, indeed, the research showed that it was all right even though they didn't follow guidelines at all. And then this second company that's bought it applies to you and furnishes the research which shows that this product is all right.

Would you have any reason not to license it?

Mr. TRACY. We would go through the whole procedure and we would review it for safety.

Mr. BEDELL. Safety—it looks like it's safe.

Mr. TRACY. It looks like it's safe. And we have basis—adequate basis—to consider that the research that was done was accurate and adequate.

Mr. BEDELL. OK. So what I'm coming to is if—if the first company had a product—genetically derived product here—it might have been dangerous as the devil to our society to release into this society. But they go right ahead and violate NIH guidelines, don't do anything about it, conduct the research in violation of that—of those guidelines and so on, and maybe expose the public to some possible terrible dangers.

It turns out they don't—those dangers don't develop. The product turns out to be safe. And then they sell it to another company, and they apply to you for a license. I take it you would grant the license.

Mr. TRACY. We'd be—

Mr. BEDELL. You would have no reason not to grant the license.

Mr. TRACY. We would go through the licensing process and we would—we would assure ourselves that the product was safe.

Mr. BEDELL. Sure. But if—

Mr. TRACY. If we were able to assure ourselves that the product was safe we would probably grant the license.

Mr. BEDELL. See the concern. It seems to me, that the problem here is that we are dealing—and I'm not sure you're at fault; I don't know who's at fault—but it seems to me we're dealing with something—and if I understand it correctly, some of these genetically derived products could present very, very serious problems to our environment, if I understand that correctly.

And that we do not have any strong controls to prevent somebody from going ahead and putting them out into our environment on their own. And you say they have peer review and so on, but they don't have to follow that peer review if they don't. And there's some indication that in this case Mr. Kit did not. Now, I'm not saying he did or didn't, but there's some question at least whether he did. And the concern, I think, exists not with what Mr. Kit has done, because I think everybody welcomes that we can do something about pseudorabies.

The issue is that it appears to me quite clear that in the procedures we have there's no real control to keep somebody from putting this out into a hog lot to try something that could be a very, very serious problem to our environment, and gamble on it, because if it works—even though they violated NIH, they can still go ahead and get their license from you. That's how I see the whole issue myself. And that's what I think we need to address in some manner in the Federal Government.

And I yield back the—

Mr. VOLKMER. Will the gentleman yield?

Mr. BEDELL [continuing]. Balance of my time.

Mr. VOLKMER. Will the gentleman yield on that point?

Mr. BEDELL. Yes.

Mr. VOLKMER. As one who has looked at the AGS matter in California and now seeing what EPA is doing, it appears clearly to me that there's a vast difference between the procedure in USDA and the procedure in EPA.

Now, I'd like to try, if I can, and you correct me if I'm wrong, going through what I see as the steps. And basically on research, and research is done under USDA auspices, whether it's through the ARS or the ACRS, whichever one, that that has to follow USDA guidelines, and those guidelines reviewed by an IBC or the ARRC. Is that correct?

Dr. BENTLEY. Yes; that's correct?

Mr. VOLKMER. Dr. Bentley?

Dr. BENTLEY. Yes.

Mr. VOLKMER. Is that correct?

Dr. BENTLEY. Yes; that's correct. And to add to it, what would be quite analogous to the process that's been very successful in NIH.

Mr. VOLKMER. OK.

But past that step, now, if it's a private industry doing the research on an agricultural plant, animal serum, whatever have you, there is no—as far as USDA is concerned—no input. They could go ahead and do lab—and do lab work—and, you know, have it filed, tested, just like anybody else, and very good lab work. And then, depending on what that lab work shows, say it looks pretty good, and they put out to some of their colleagues, and they review it and they say that's fine. They could then, Dr. Tracy, as I understand it, go out and do a field test with that. Let's say we're talking about something like we'd like to get rid of in our part of the country—and I know a gentleman in Iowa, nematodes on—for soybeans—and I developed a seed; I can tell people I've done it and all this. And we do the lab work and then we go out and put it in the ground. Nobody is going to stop me from USDA—not one person. Correct me.

Mr. TRACY. Well, not under the licensing procedure. I don't know—

Mr. VOLKMER. Not under any procedure I know of—nobody's going to stop me.

Now, let's say that I decide I'm going to put this plant—the seeds in the ground in cooperation with the University of Missouri Cooperative Extension Service, and they don't bother to notify Dr. Bentley. And I go ahead and do it in cooperation with them. And they verify that it is nematode resistant; we've planted this thing 2 years in a row.

Now, Dr. Tracy, I'm the private company and I'm coming up to you, and I have the University of Missouri Cooperative Extension, and maybe I've even gone to Ames and got the University of Iowa or Iowa State, one of the two—they have good research facilities. And they say, yeah, it does too, it really does this. And, Dr. Tracy, you are going to say, hey, that thing is efficacious, it's safe, it's everything else; it's all proven up; sure, go ahead and do it. Aren't you?

Mr. TRACY. You are referring to the release of the plant and not the—

Mr. VOLKMER. Yeah, the seed, the use of the seed.

Mr. TRACY. Right.

Mr. VOLKMER. Sure. You're going to say that.

Do you know what you're telling me? You know, if I was Calgene today what I would do? I'd go out and plant my tobacco plants. That's just what I'd do. But nobody is going to stop me from plant-

ing those tobacco plants anywhere in the United States—only I'd find me Kentucky University or North Carolina, or someplace else, to cooperate with me in planting those tobacco plants—just like Dr. Kit did with Texas A&M people and with the Texas people. And I'd go out and put those tobacco plants in the ground.

And after its all proven up, those tobacco plants are all right, Dr. Tracy, I come in to you. I'm Calgene with these proven tests that I've done out in the field. And if they've proven up through the University of North Carolina and University of Kentucky, and others, that is fine. You are going to go ahead and license it, aren't you?

Mr. TRACY. If you complied with all requirements for plants—under Plant Pests Act, yes. You're not talking about a plant that would be a pest here. You're talking about—

Mr. VOLKMER. No, this is—

Mr. TRACY [continuing]. Using a nematode in your testing procedure.

Mr. VOLKMER. Well, Calgene's got a tobacco—varying tobacco that gets rid of some of the tobacco disease—genetically engineered.

Now, Calgene, as I understand it, because of what's going on in USDA, has just recently withdrawn their request for approval now. Given up for the time being. Is that right, Dr. Bentley?

Dr. BENTLEY. No, not so far as I know. They have asked for a delay in that—but, and, I guess pending the outcome of these discussions—I haven't talked necessarily directly with them. But the point of it is when you mention in the case of Missouri—I'm sorry.

[The material follows:]



CALGENE

May 2, 1986

Hon. Harold Volkmer, Chairman
Subcommittee on Investigations
and Oversight
Committee on Science and Technology
2123 Rayburn House Office Building
Washington, DC 20515

Dear Congressman Volkmer:

I am writing to you because of what may be a misunderstanding, arising out of yesterday's joint Science and Technology and Agriculture Committee hearing, about the current status of Calgene application to USDA for approval to conduct a field trial of genetically engineered glyphosate resistant tobacco plant. While Calgene's field trial application was not the subject of the hearing and consequently, Calgene was not a witness, I would nevertheless appreciate it if this letter could be made a part of the hearing record.

At the time Calgene decided to seek USDA approval for its proposed field trial we were aware that USDA (and the other regulatory agencies) had not as yet articulated clear guidelines on how the agency would process applications for field trials of genetically modified organisms. While we believed (and continue to believe) that our experiment poses no risk to the environment, we were also mindful of the potential for public misunderstanding about new technologies such as biotechnology, and therefore, decided at the outset to publish or otherwise make available to the public the data supporting our application. We also went to great lengths to inform state and local governments to be open in public discussions of our proposal and to publish the results of our work in the scientific literature. In testimony before your Subcommittee on December 4, 1985, Dr. Robert Goodman, Calgene's Vice-President for Research and Development, submitted for the record and described in detail Calgene's proposed field trial.

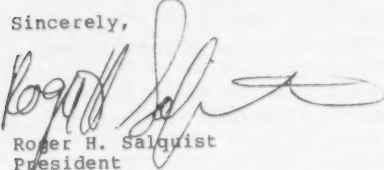
Congressman Volkmer
May 2, 1986
Page -2-

While some have suggested that Calgene is not required to wait for USDA approval to conduct its field trial, we decided, notwithstanding questions about USDA's jurisdiction, that we would all be better served if, at this early stage of commercial development, the USDA process proceeded to take its course. While we are disappointed that the regulatory pathway at USDA still remains cloudy, and that recent events have raised questions about the USDA approval process, we have not commenced our field trial, and have no present intention to start the trials without USDA approval.

Recently we made a decision to defer submission of final pre-trial data to USDA in order to put our application on hold; we have not withdrawn our application from USDA. We intend to submit our data to USDA after the biotechnology procedures, which have been under review at all the relevant agencies since January 1985, are published, and the other issues surrounding USDA's jurisdiction are satisfactorily resolved.

I appreciate this opportunity to clarify the record and, if I can provide any additional information, please have your staff contact me.

Sincerely,



Roger H. Salquist
President

RHS:cbh

cc: Hon. Berkley Bedell, Chairman, DORFA
Hon. Ron Packard, RMM, Investigations and Oversight
Subcommittee
Hon. Pat Roberts, RMM, DORFA

Mr. VOLKMER. Well, let me finish what I started out with—that the difference between you and the EPA is before I can get in there to EPA to get me a license, Dr. Tracy, OK?—I've got to get me an EUP before I can do a test out there on a field somewhere.

Now, you don't have anything in Ag that makes me come to you, to any scientific advisory group, approval or anything, to go out and do a field test. There's nothing within the USDA.

Mr. TRACY. If there's any prospect that that plant might be a pest, there is a procedure that you would have to go through.

Mr. VOLKMER. The plant be a pest? You mean if a plant is—

Mr. TRACY. If the plant itself was a pest, yes. I think in your example you're talking about, in effect, releasing the—the nematode. But when you were talking about licensing the plant and the risk that might come from releasing the nematode in the process of—of testing the plant—

Mr. VOLKMER. That's correct, testing the seed for it.

Mr. TRACY. Well, the research that would be conducted on the risk of releasing that nematode would be under research guidelines. The licensing of the plant would be based upon whether or not the plant was or had the potential for becoming a pest.

Mr. VOLKMER. But the research guidelines do not prevent me—under USDA—from going in and doing it. That's my problem. No one does. Not under private research.

Dr. Bentley.

Dr. BENTLEY. That's—I think that's essentially right under private research they would do. Of course, that places the person that does it under some jeopardy in case of problems. But I think that we are seeing—that we anticipate that the industry where it's privately funded, will want to comply with the general guidelines—much as that's been the experience in the case of the biomedical research primarily in the NIH guidelines. We would hope that that would be a compliance, and I would think that—I would anticipate that they would.

Mr. BEDELL. Would the gentleman yield?

Mr. VOLKMER. Yes; I yield before I make my closing statement and call the final panel.

Mr. BEDELL. Why? Why would they bother?

Dr. BENTLEY. I think because of the credibility question, they would bother.

Mr. BEDELL. The what?

Dr. BENTLEY. The credibility. That is, they would want it through this. I don't know what kind of exposure that would place a company in.

Mr. BEDELL. But if they don't bother, they speed things up and help themselves financially, don't they?

Dr. BENTLEY. Well, yes, they might. But they could also, if the things didn't work out as well as we have used in our example, I don't know what that does to their liability. And I would think that would increase. I don't know, but I would think it would.

Mr. VOLKMER. Well, let's say that things went wrong instead of going right—

Dr. BENTLEY. Yes.

Mr. VOLKMER [continuing]. Then in some—something that is released into the environment, some harm comes to a bunch of farm-

ers, or what have you, all over, I don't know how you are going to prove it was caused by that experiment. That may be a little difficult—causation.

I am more worried about something else. What concerns me, Dr. Bentley, and the reason we have had some of these hearings is because, you know, back several years ago, there were those in the Congress—and I would say outside the Congress—who were concerned about the biotechnology field. And many proposed that we have legislation—statutory—more controls, et cetera, in this whole area. And as you have mentioned, the Scientific Policy Committee and adviser came up with this idea of what we are following now.

Now, there are other people getting worried about what we are doing now. And what I see is more of a push to go back to many more stringent controls. And what I'm seeing from you, and you are telling me here today—to be honest with you, I'm going to have to discuss it with the Secretary because I like the idea—first place, of improvements coming along through biotechnology and being able to utilize, as long as it's safe—

Dr. BENTLEY. Yes.

Mr. VOLKMER [continuing]. For—and not help prevent—causation to health problems or anything—for the agriculture community, and anything else, but especially agriculture. And I think it shows a great promise for the future. But what I am seeing is the possibility—and I think the gentleman from Iowa sees—there's—you've got a loophole right through here where you can drive a big truck. And people can go out there and do about anything they want to do if they've got the money to do it with, and you are not going to do anything about it in your field.

Dr. Tracy, you are not going to do a thing until somebody comes up and asks for a license.

Mr. TRACY. Well, there is—I don't know all the details of it, but there is a plant pest act which has some restrictions against—

Mr. VOLKMER. Sure, and we've got the weed control act and that type of thing that has some restrictions on it.

Mr. TRACY. Prohibitions against bringing things in from foreign countries and introducing new—

Mr. VOLKMER. Exotic type of things. But these aren't exotic. We have no procedure actually in the biotech field for making sure that people, when they conduct field tests, do it under some, at least control, or at least some review by somebody else before it's done.

I know it's not your job.

Mr. TRACY. Well, if products have the potential of being pests, there are requirements before they can be field tested under the Plant Pest Act.

Mr. VOLKMER. I asked Dr. Jordan this question

So if some company out there, an individual, wishes to test a biotechnology altered plant, they could do so without your knowing anything about it?

Dr. JORDAN. It would be possible. It wouldn't happen for very long without us learning about it shortly.

Mr. VOLKMER. Would it be possible to initiate it and they would have violated no law?

Dr. JORDAN. That's correct, sir.

Mr. VOLKMER. No regulation; is that correct? Dr. Wood said, "No, sir, that's not correct."

And then he gets into the question of the pests and the weed.

But then the problem is—what I see is, if I do the testing and it does turn out supposedly all right, that has—even though I haven't followed any governmental outlines, Mr. Tracy, when I come with a proposal to you for a license, you are really not concerned of whether I followed any guidelines as long as there's basically peer review or approval by some other bodies of what I've done, and proving up the safety of it, and the efficacy of it—whether it's through a university, a private group, a scientist, or what—that's going to be satisfactory to you, is it not?

Mr. TRACY. If you had, in the process, field tested a potential plant pest you would presumably have been in violation of the law.

Mr. VOLKMER. Well, would that make any difference to you?

Mr. TRACY. Of course.

Mr. VOLKMER. Would you turn down the license?

Mr. TRACY. We would review each license application based on its individual circumstances. I would certainly be rather unimpressed by someone who violated the law in his testing procedures.

Mr. VOLKMER. Wouldn't it be a little better if there was a provision in the regulations before a person could in the—this genetic engineering field, provide that before they could do a field test that they would have to submit data, background, laboratory, et cetera, and a proposal how they are going to do it to you before they did that?

If they want to—if they are going to come up to you for a license, that they would have to do that beforehand?

Mr. TRACY. Just a second.

[Pause]

Mr. VOLKMER. What I'm basically asking you to do, is what EPA does.

Mr. WALSH. Mr. Chairman, if I might, under the plant pest statutes we have new regulations in the process now which we expect to ready in a short while which will govern the issuance of permits and regulate the field testing of potential plant pests.

Under VST, we already have that set of regulations. We already have. There's an entirely different process. And under the VST statute, now we are dealing with—with veterinary biologics.

Mr. VOLKMER. Right.

Mr. WALSH. So that the field testing that took place under the Virus-Serum-Toxin Act was done with the permission and with the approval of the Veterinarian Services staff at the Animal, Plant Health Inspection Service.

So there are two—two different topics we are dealing with. And I think that's leading to some conclusion—some confusion as well.

Now, it's true under VST, we do not have jurisdiction over the testing that took place prior to the license application. In other words, for that veterinary biologic—that's true. At that time, we had no jurisdiction.

Mr. VOLKMER. At that time?

Mr. WALSH. That's right.

Mr. VOLKMER. What about the future?

Mr. WALSH. Well, now we have jurisdiction over the intrastate movement and use of these things as well. So that it could be that in the—that in that process, for example, the movement of that—of

that vaccine from Baylor to Texas A&M, that that would be subject to regulations, which are not in place yet, but which are being developed now by the Animal, Plant Health Inspection Service. That's under the—the Food Security Act of 1985—the amendments.

Mr. VOLKMER. What about vaccines that are to be used in a field test? Would you cover those?

Mr. WALSH. Well, it could be, I don't know exactly what the staff is going to do with that, sir. And I don't know how they are—exactly how they are approaching it. But certainly they have—they have some control over the movement of those intrastate as well as interstate. And now, once the licensing procedure starts, of course, we approve and review those requests to field test potential licensed products.

Mr. BEDELL. Will the gentleman yield?

Mr. VOLKMER. Yes, I yield to the gentleman.

Mr. BEDELL. Very shortly. I want to get this on the record.

Did the USDA check to see if the test involving the 1,400 pigs was done in compliance with the Agency and NIH/RAC guidelines? Dr. Tracy, did you check to see?

Mr. TRACY. As part of the review process afterward, I would have to check.

Mr. BEDELL. Couldn't hear you.

Mr. TRACY. As part of the licensing review process, the licensing review process took place after that test.

Mr. BEDELL. Yes. And my question is: As you reviewed it for the license, did you check to see if those—if that test was conducted under NIH agency, NIH and RAC guidelines?

I think the answer is "No" but I want to get it on the record.

Mr. TRACY. We will have to check and give you a specific answer. I don't know whether we checked on that specific test to see whether it complied with NIH guidelines. It was really more of a research question.

Mr. BEDELL. So not necessarily then?

Mr. TRACY. Correct.

Mr. BEDELL. Is that correct?

Mr. TRACY. Yes.

Mr. BEDELL. Thank you.

Mr. VOLKMER. Well, thank you very much, Dr. Bentley and Mr. Tracy. I think you understand, I hope, at least my concerns—I think several members of the committee and others—about the field of—of this genetic engineering and recombinant DNA field. And basically, not to deny the continuation of it and the growth of this field, but to make sure to the public the health and safety features, both to them and to the environment.

Dr. BENTLEY. Thank you, sir. I go back to my statement—that's a concern of the Department.

Mr. PACKARD. Just—

Mr. VOLKMER. The gentleman from California.

Mr. PACKARD. Just simply on the question that I asked regarding the February 7 letter. I would like to have that letter submitted to—for inclusion into the record. It does clarify—

Mr. VOLKMER. That's the letter from—

Mr. PACKARD. It's the letter from—from Randall Levings to Mr. Shibley.

Mr. VOLKMER. All right.

Mr. PACKARD. It does clarify the question that I asked regarding the purity and identity tests.

Mr. VOLKMER. All right. That will be made a part of the record at this point in the record.

[The letter follows:]



United States
Department of
Agriculture

Animal and
Plant Health
Inspection Service

Veterinary
Services

National Veterinary
Services Laboratories
P.O. Box 844
Ames, Iowa 50010

Subject: Testing for License No. 272

Date: February 7, 1986

To: G. F. Shibley
Chief Staff Microbiologist, VS/RPPS/VBS
Federal Building, Room 833
6505 Belcrest Road
Hyattsville, MD 20782

The additional testing has been completed on the MSV (LS200-1001) for Lic. No. 272, Product Code 18V1.80, PRV recombinant vaccine. I regret that cell supply problems delayed both components of this testing.

One component of the testing consisted of restriction endonuclease analysis of the MSV + 1 vs. a BUK strain sample of independent origin. The appropriate lanes from one agarose gel electrophoresis run are shown in the enclosed figure. The results observed using the restriction enzymes Bam HI and Kpn I are shown because of their direct bearing on the nature of the construct. As can be observed in Figures 2, 3 and 4 of the patent (#4, 514, 497), it was a specific Bam HI fragment #11 which was engineered for deletion. This segment 11 is shortened relative to BUK, while all other Bam HI fragments should be the same size. This is shown clearly in the enclosed figure as well as figure 3a of the patent. Similarly, the Kpn I fragment J₁ is shortened relative to that of BUK. Again, similar results are shown on the enclosed figure and figure 3b of the patent. These results are consistent with a PRV constructed as outlined in the patent and supplemental information, i.e. a deletion (in Bam HI fragment #11) of PRV (BUK strain).

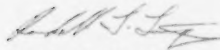
The other component of testing was accomplished by Mr. Mike McFarland, a PhD student volunteer in our laboratory, who has recently completed a revertant-selection assay similar to that described by Campione-Piccardo et al. (1979, J. Virol. 31:261) and noted by Kit et al. (1985, Am. J. Vet. Res. 46:1359). In brief, 10^5 pfu (≈ 10 doses) of MSV+1 or a TK+ PRV control was used to infect L-TK- cells in the presence of HAT (Hypoxanthine, Aminopterin, Thymidine) media. This should allow only TK+ PRV to be amplified. After three passages (at 3 day intervals) in such cell cultures, virus was assayed on MDBK's without HAT media. No virus was observed from the TK- PRV (MSV+1) origin. Virus was observed from the TK+ PRV control origin. The conclusions that can be drawn from this assay are that the MSV+1 contains less than one contaminating TK+ virion/ 10^5 virions, or that less than one reversion occurs for each 10^5 parent virions.

I feel that these results further establish and confirm the purity and identity of the PRV MSV NS200-1001. It obviously would have been preferable

G. P. Shibley

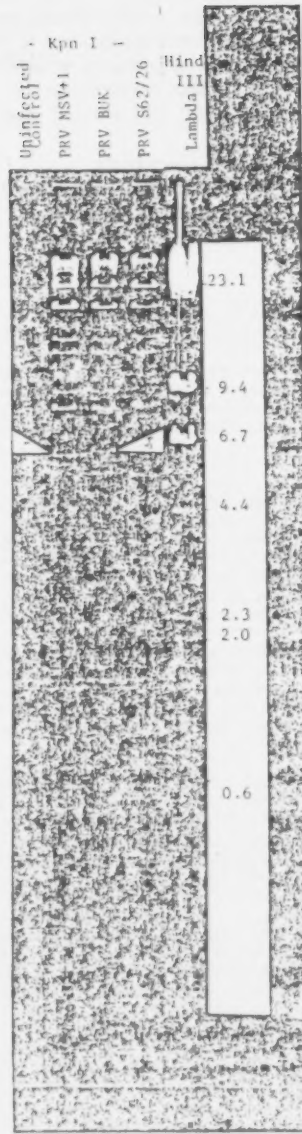
2

if this data had been submitted prior to licensure. However, I hope this information will be of some use to you at this time.



Randall L. Levings
VMO, Large Animal Section
Biologics Virology Laboratory

Enclosure



Mr. VOLKMER. Yes, I would, Mr. Tracy, appreciate, after you have reviewed what you did do in regard to the Texas test of the 1,400, that you submit that in writing as to whether or not you came to the conclusion that it did or did not meet NIH or even look at that as to whether—whatever you did in regard to that—and submit it to the committee for purposes of insertion in the record.

We will hold the record open. Would 1 week be sufficient, Mr. Tracy?

Mr. TRACY. Certainly.

Mr. VOLKMER. One week for that purpose.

[The information follows:]

DEPARTMENT OF AGRICULTURE
OFFICE OF THE SECRETARY
WASHINGTON, D.C. 20250

May 8, 1986

Honorable Harold L. Volkmer
Chairman, Subcommittee on Investigations
and Oversight
Committee on Science and Technology
House of Representatives
Washington, DC 20515

Dear Mr. Chairman:

At the April 29, 1986, hearing on the research, field testing, and licensing of the recombinant derived pseudorabies vaccine, you asked about the field test of the vaccine conducted on the Maddox swine herd in Texas. Specifically, you asked us to determine whether the Animal and Plant Health Inspection Service (APHIS), during its processing of TechAmerica's application for a license under the Virus-Serum-Toxin (V-S-T) Act, had reviewed the use of the vaccine on the Maddox herd for compliance with the National Institutes of Health (NIH) research guidelines.

TechAmerica's license application was filed with the Department on December 27, 1984. You should note that the Maddox herd inoculation was not conducted by TechAmerica, the license applicant. The Maddox herd was inoculated in June 1984, at which time the Secretary did not have jurisdiction under the V-S-T Act to supervise or regulate such a test. The Food Security Act of 1985 amended the V-S-T Act to give the Secretary jurisdiction over intrastate as well as interstate shipments of veterinary biologics subject to certain exemptions. APHIS is currently developing regulations to implement the 1985 V-S-T amendments. Pursuant to the animal quarantine laws, the Secretary has the authority to take such measures as are necessary to prevent the introduction or dissemination of infectious or communicable animal diseases. The additional authority conferred by the 1985 V-S-T amendments together with this existing authority under the animal quarantine laws should enable the Department to deal effectively with the issues raised in connection with the inoculation of the Maddox herd.

We pointed out in the environmental assessment prepared by APHIS in connection with the licensing of TechAmerica's Omnivac vaccine that data from studies conducted prior to the application, including results of the Maddox herd test, were submitted as background information by TechAmerica. All such data were considered by APHIS officials in the licensing process. APHIS officials did not, however, review the Maddox herd inoculation for specific compliance with NIH research guidelines.

The V-S-T Act declares unlawful the shipment of veterinary biologics that are worthless, contaminated, dangerous or harmful, and prohibits the shipment of any veterinary biologic unless it is prepared in compliance with regulations prescribed by the Secretary at a properly licensed establishment. The V-S-T

Honorable Harold L. Volkmer

2

Act and the regulations issued thereunder impose vigorous requirements on license applicants. Applicants for a product license are required to demonstrate that the product is pure, safe, potent, and efficacious before a license can be granted. The first step in the licensing process is submission of a product license application (Form VS-14-3). Supporting data are compiled and submitted by the applicant throughout the licensing process. Data required by the regulations include an outline of production test reports and research data sufficient to establish the purity, safety, potency, and efficacy of the product, legends designating which facilities are to be used in product preparation, and printed labels which include any information and advertising to be placed on such labels. All studies and tests conducted by the applicant after filing of the application are accomplished under USDA supervision.

TechAmerica scrupulously followed the prescribed licensing procedures and satisfied all information requirements. At the time TechAmerica filed its license application, it also submitted a backpassage protocol for APHIS review designed to demonstrate that the avirulent properties of the virus were stable. This was the first step toward USDA approval of TechAmerica field studies outside its laboratory. As with all license applications, TechAmerica was required to submit to APHIS a field study protocol for the vaccine. APHIS officials thoroughly reviewed this protocol and only then permitted the applicant to conduct testing. Protocols for additional field trials were submitted and evaluated at each step in the licensing process. Additional testing was also accomplished at the National Veterinary Services Laboratories in Ames, Iowa. The safety, purity, potency, and efficacy of the vaccine was amply demonstrated by this licensing procedure, and in accordance with V-S-T Act, the Secretary was required to issue the license to TechAmerica.

Sincerely,



Alan T. Tracy
Acting Assistant Secretary
Marketing and Inspection Services

Mr. VOLKMER. With that, that will conclude this panel, and we will now go to the third panel. I appreciate Mr. Jeremy Rifkin, president of the Foundation on Economic Trends, to come up to the table.

Now, your statement, which has been made a—or submitted to the committee has been reviewed and will be put in the record in full. You may either review the statement in full or you may summarize, however you so desire. Mr. Rifkin.

STATEMENT OF JEREMY RIFKIN, PRESIDENT, FOUNDATION ON ECONOMIC TRENDS

Mr. RIFKIN. Thank you, Congressman.

I am going to let the testimony stand on the written record. I am not going to read it. I have specific comments as a result of today's hearing.

First of all, I would like to let members of this committee know that this morning, my foundation sent—our legal counsel sent a memorandum to the head of the National Institutes of Health and to the Secretary of Agriculture requesting that—of the Secretary of Agriculture, an immediate suspension of this license once again pending a full-scale investigation by the National Institutes of Health and other Government agencies as to possible violations by the Baylor College of Medicine and NovaGene in regard to the National Institutes of Health Guidelines governing this research.

Let me try and place this within two contexts. The first part of this process had to do with the field testing in Texas with Baylor College and NovaGene. The second part of the testing, as you've brought out in this testimony has to do with Biologics Corp.'s application to USDA.

We believe there have been serious violations of Federal statutes in both regards.

First, let me go to the first set of tests. There was a joint venture. Let me first point out that under the National Institutes of Health guidelines, any institution that receives Federal funds must go in front of the National Institutes of Health Recombinant DNA Advisory Committee for approval of genetically engineered microbe field tests.

Now, in relation to the Baylor College of Medicine, a contractual relationship was entered into between NovaGene and the Baylor College of Medicine in which the Baylor College of Medicine would provide facilities and other resources to perform—control tests in the laboratory, in return for which both the Baylor College of Medicine and NovaGene would be joint owners of the patent. This was a joint venture.

Therefore, when NovaGene developed a relationship with Texas A&M to do field testing in the State of Texas, it is my understanding that since this is a joint venture between the Baylor College of Medicine and NovaGene, that this made the Baylor College of Medicine responsible and potentially in violation of Federal laws. As I said, the NIH laws require that any institution that gets Federal moneys has to go in front of them for approval. And Baylor College of Medicine has gotten Federal funds and so has Texas A&M.

So it seems to me here on the surface of it, we have a clear violation of the NIH statutes here that do govern the first part of this process. In the sense that the NIH is empowered to—if there is a violation and if the university that is involved in this research has not come in front of the NIH/RAC Committee, the NIH is empowered to take away all Federal funds to that institution. In this case, the Baylor College of Medicine and perhaps Texas A&M for their involvement in the field testing of this without appropriate—without the appropriate procedures being used at the NIH.

Now, the second stage in this process has to do with the Biologics Corp.'s proposal in front of the U.S. Department of Agriculture. And if I may, let me digress for a moment and explain to this committee how these documents were made public, because I think they—they speak to the lack of regulatory oversight.

I received a series of confidential memos beginning in November 1985 and all the way up through last week—April 1986—purportedly from one or more people from another Federal agency. And these memos informed me that—that NovaGene and Baylor were involved in surreptitiously moving a genetically engineered deliberate release experiment to the Animal and Plant Health Inspection Service in violation of Federal regulations without the knowledge of other agencies.

As a result, through the Freedom of Information Act, we secured documents which we have made available to the committee and to the public, which in my mind clearly shows that there was an effort on the part of NovaGene, at least, to make sure that their field test did not get the appropriate review of the National Institute of Health. And, rather, I think they thought they would have a much more sympathetic audience at the Animal and Plant Health Inspection Service, and I think the case that—that case has been borne out.

Let me point out a couple of problems—and I am going off on a few tangents. But one thing that really—I thought it was a *deja vu* here this morning on what is a contained facility. Congressman Volkmer, you remember last time that AGS was up here as a result of documents that we disclosed about their wrongdoing, the company said that they thought a tree was a contained facility. And after the appropriate discussion, the Environmental Protection Agency informed them that a tree in the environment is not a contained facility.

Now, both NovaGene and Biologics Corp., are saying that a pig is a contained facility; that it is not in the open environment.

I point out that even the cochair person of the USDA RAC Committee, Sue Tolin, who has been involved with this process as an agency representative on the NIH/RAC Committee for years, is quoted in the press as saying, of course she would have told them that a pig is definitely not a contained facility. It is a deliberate release experiment.

As to the question of whether viruses shed and spread, I would like to point out to this committee that a meeting was held by the AAA and the Environmental Protection Agency last year on the question of releasing genetically engineered viruses in the environment. Those proceedings have been published. I have provided that publication to your staff counsel on the committee. And in these

proceedings the scientists that were involved at AAA's and EPA pointed out that there is going to be some potential problems with releasing viruses in the environment and that we have no clear protocols and no clear understanding of the science of environmental fate in the case of viruses. And that we need to develop very hurriedly some protocols and some procedures for—for analyzing the risks involved to the environment of placing viruses out there.

So, obviously, viruses are a deliberate release. Obviously, a pig is not a contained facility anymore than a tree is a contained facility. I think that's stretching—stretching it a bit much.

The—we have, as you know, a lawsuit pending. I don't want to get into that here in the committee hearings, but we have sued the U.S. Department of Agriculture because we believe they have violated their own statutes. The USDA has been on record for years as saying they would adhere to the NIH guidelines. They have set up a formal review committee—the ARRC, the Recombinant Advisory Committee—to oversee genetic engineering experiments. As late as last year, they informed the Government Accounting Office—and it's in the GAO report on USDA—they informed the Government Accounting Office that when they got to the point of dealing with live genetically engineered viruses that they would develop more rigorous procedures of oversight and run it through their formal regulatory process at the RAC Committee. That's on page 48 of the GAO report, I believe.

Now they come in front of your committee this morning and say, well, that's not really the case. The RAC Committee, it appears, is just a weak advisory committee. And I think that might be the case because the GAO has pointed out that that committee has met a total of 15–20 hours in 15 months. I hardly believe that's rigorous oversight for a lead agency involved in genetic engineering technology.

There's a real question of a dual standard of justice here. And I think that Congressmen and members of the committee have been pointing to this all morning. I don't mean to be redundant but here's Advanced Genetic Sciences—a company in California. It illegally places microbes into the environment before it gets permission to do so by a government agency. And it falsifies data in regard to pathogenicity studies.

The EPA says they violated the law. They suspended their license. They fined them \$20,000 for their wrongdoing.

Then case two. Here you have Baylor College of Medicine and NovaGene violating the National Institute of Health regulations which require that they bring a deliberate release experiment in the research stage before their committee. And then, second, you have Biologics Corp., going in front of USDA, and the USDA decides not even to run it by their formal review process.

It seems to me that if AGS can be fined for violating Federal laws, why is the USDA above the law?

Why are NovaGene and the Baylor College of Medicine above the law?

Why is Texas A&M potentially above the law?

Now, it might prove, in the final analysis, that there is no wrongdoing here. I feel that that's not going to be the case, but certainly it warrants a full investigation by the appropriate agencies. And it

seems—what I have asked the Director of NIH and the Secretary of Agriculture to do this morning in a formal request, is to revoke this license once again, to suspend it until such time as there's an investigation by the NIH about wrongdoings of its policies by Baylor College and NovaGene, and Texas A&M, potentially; and until there's a full investigation by the USDA of potential violations of its policy.

It seems to me that's fair and that's equitable. And, otherwise, we will have a double standard where some companies and private institutions, and Government agencies can go scot free for potential violations, whereas, other agencies and private companies, and universities, have to adhere to a code of ethics and a code of law.

The last thing I want to talk about here has to do with—by the way gene deletions. The argument that one gene deletion doesn't fall under the guidelines is just pure silly. There is nothing in the guidelines which says that if you only delete a gene it is exempt from the guidelines. In fact, as the committee knows, the Frostban bacteria is one gene deletion, and that had to go through the entire regulatory process of the National Institutes of Health. And it was also subject to the full and rigorous environmental process of the Environmental Protection Agency.

Here you have a virus with one gene deletion. And there's nothing in the regulations that exempts it because it's a simple gene deletion.

Finally, in regard to this technology overall, there is no doubt that there is—there are benefits to be derived from genetic engineering technology.

I also think, however, it's disingenuous and naive on the part of certain members of the scientific establishment and private industry to say there's no potential environmental risk. The reason is this: Genetically engineered products, when you place them in the environment, are distinctive from petrochemical products in three ways—one, they are alive; two, they can reproduce, mutate, and grow; you can't constrain them as easily; and, three, you can't recall them. If you don't like what a microbe has done out there in the environment, you can't seal it up, you can't clean it up, you can't take it back to the laboratory.

Now, the rule of thumb that we have used throughout this entire controversy over the past many years is this: Genetic engineering introductions are low probability, high occurrence risks. Meaning that whenever we place one of these microbes into the environment there's only a small chance something is going to go wrong. But if something does go wrong, the event can be subtle, but in the long run, it can be enormous and potentially catastrophic.

The analogous situation being the introduction of exotic organisms. We brought a lot of organisms over to North America from all over the world. Most of them had been benign. A small percentage of them, however, have proved to be pests, like Dutch elm disease, and chestnut blight, and gypsy moth, and kudzu vine, et cetera.

So I think that when we deal with regulations in developing new legislation, we have to understand that genetically engineered products are not going to be immune from environmental problems. And I think that the Congressmen have all spoken eloquently

to that this morning. They are not all going to be a problem, but some of them are going to be a problem. And we need to develop the appropriate rigor and oversight so that we don't repeat the mistakes of the past.

I grew up in the 1950's. And in those years we did not ask the hard questions about potential environmental problems associated with nuclear power technology and petrochemical technology. We are enamored with moving ahead with this technology and getting it on line, and we didn't ask the hard environmental questions up front.

As a result, we have had some benefit from these technologies, but we have also developed a tremendous—environmental bill—which future generations have to pay for. The best example being evident. We take a look at the nuclear power accidents here in this country and now in the Soviet Union, and examples ranging from acid rains to nuclear waste dumps.

We don't have to repeat this mistake this time. If we take a look at the hard questions up front, perhaps we can develop a rational approach to decisionmaking in which the environment is protected and human health is protected in the process.

Mr. VOLKMER. Mr. Rifkin, I don't—I hate to interrupt but we have a vote on and we are going to have to leave, and we will come back in about 10 minutes. We will recess for 10 minutes. At that time you can conclude your statement and we will have questions at that time.

[Recess.]

Mr. VOLKMER. The subcommittee will resume.

Mr. Rifkin, you may conclude your statement.

Mr. RIFKIN. Well, there's one other thing I notice in looking over my notes, Congressman Volkmer, is that I find it interesting that Calgene—the Calgene proposal went in front of the RAC Committee. Calgene is a private company. It went through the RAC Committee process at the USDA for a potential field permit.

What's the difference between that and Biologics Corp.?

Biologics Corp., first applied for a field permit and then second a commercial license.

I fail to understand what the distinction is between one and the other.

I'm—I'm as lost as you are after 3 hours of this. I—it's rather—what it really indicates to me here is that the regulatory process means whatever the agency wants it to mean, whenever it wants to get on with the job of putting the products out in the environment. And it changes its story every 48 hours. It's hard to keep in touch with what the agency is saying.

Mr. VOLKMER. That concludes your statement then, Mr. Rifkin?

Mr. RIFKIN. Yes.

Mr. VOLKMER. OK. Now, I'd like to ask a couple of questions.

What review do you feel is required under the court decision in the case of Foundation on Economic Trends versus Health and Human Services?

Mr. RIFKIN. Well, that was—that was quite interesting, on the original Frostban experiment. And, again, we have a case of direct analogy in—and, if you will, *deja vu*. The National Institutes of Health—we argued in court—that the NIH did not do an environ-

mental assessment as required by the National Environmental Policy Act.

The NIH went to court and said they had done an equivalency of an environmental assessment and they had done the paper work but they hadn't labeled it that.

The court ruled in that case, Judge Sirica—the District Court for the District of Columbia—ruled that you can't, after the fact, just shuffle some paper together and call it a formal environmental assessment. And you can't just make conclusory statements. You have to do an environmental assessment. So the NIH was required to go back and do one.

I should say we were upheld in that decision by the appeals court—Federal appeals court—in the District of Columbia.

What surprised me about the suspension of this license by APHIS was this: They had a chance to go—go ahead and do it the right way the second time around and they still did it the wrong way.

I found it unconscionable that Mr. Hawkins, who is head of APHIS, said in print, that all he needed to do was "develop a paper trail." To my mind, and someone who is involved in the environmental movement, that is rather an unconscionable statement. It suggests that he is not really concerned about the regulations of NEPA and sees them as a nuisance and something that he merely has to provide some paper with in terms of a paper trail.

The environmental assessment has to be done before the licensing, not after the fact.

The other thing that concerned me about this is the—the assessment that was done—I am sure you have read that assessment, Congressmen—and in that assessment they say that, at USDA, one of the reasons they find this safe is they went back to Saul Kit's original test in Texas on his herd. And they found those tests to be safe and that was used as their background material to help provide the environmental assessment.

In other words, they relied on those initial tests in Texas as a basis for making part of their judgment as to the efficacy and safety of this commercial licensing under the National Environmental Policy Act.

Well, as it's been pointed out today, and as we pointed out this morning in our memorandum to the National Institutes of Health and the U.S. Department of Agriculture, there's reason to suspect that these initial tests done in Texas were in violation of Federal laws, the NIH guidelines. And they were done in violation of those laws placing Baylor College of Medicine, NovaGene, and Texas A&M in the position of possibly disobeying Government statutes.

It seems to me for the USDA to then do an environmental assessment after they put this product out and rely on data that might have been gathered illegally, is something rather extraordinary. I don't know of any—any precedent for that in government policy where an agency does an assessment after the fact based on research that might have been gathered illegally. But I am not a lawyer.

Mr. VOLKMER. All right.

The gentleman from—well, one other—one other thing, I guess in general, Mr Rifkin.

Is it your general position that any product derived from a biotechnology process—in other words, procedure—or through a genetic engineering, or what you call recombinant DNA—that any such material is—should not be able to be released?

Mr. RIFKIN. My position all along has been this: There are benefits to this technology and there are risks. In some cases, the short-term benefits seem extraordinary. In other cases, the long-term risks definitely make part of this technology problematic.

You might be aware of the fact, Congressman, that I have not specifically critiqued some parts of this technology. There has not been any criticism or any open attempt to stop pharmaceuticals as it relates to genetic engineering. We have never had a position critical of the use of genetic engineering technology with pharmaceutical products. By and large, I consider the pharmaceutical products not to be of the same level of risk as living organisms.

Now, there might be some problems with specific pharmaceuticals as a result of genetic engineering technology. But as a generic category, I have never raised any objections to pharmaceuticals and that's the main area where genetic engineering is now on line in terms of product development.

I have raised questions about deliberate release. I think it is a big, long-term question for civilization. And if we don't deal with it now, we are going to have big problems 100 or 200 years down the line when we have thousands and thousands of genetically engineered microbes criss-crossing this continent and around the world, some of which are going to be real pests.

I have raised questions about genetic engineering in biological warfare. And we have been engaged in lawsuits with the DOD, as you know.

I have raised questions about one aspect of human genetic engineering only: germ line therapy.

I have not raised criticisms about the ethical implications of somatic therapy.

So I would like the record to show that through these years we have had a selective approach to genetic engineering as a broad technology. I think there are big problems with it.

I think there are long-term problems when we start a journey where we begin to engineer genetic changes in the code from microbes, plants, animals, and humans. In fact, I know of no more impressive social question before civilization in the coming centuries as to whether we become the architects of parts of the genetic code of life that raises serious ethical, social, economic and environmental questions. And I think we should not skirt—skirt those questions. We ought to deal with them head-on now rather than later.

Mr. VOLKMER. I assume that you had firmly believed that before there could be any field testing of any product or genetically engineered—whether it was recombinant DNA or others—that would have to go through some type of review process by scientists for approval before it ever could be field tested for release.

Mr. RIFKIN. I think it should. And I have also—I have learned a lot in the last few years about how things sometimes work in this city. I have been, for example—it has been quite interesting to me that on the one hand, in terms of regulations, we have government agencies who have done panels, and study commissions, and re-

ports, and we have had congressional hearings, where there's been an acknowledgement that we have no science yet to judge the risk of releasing genetically engineered products in the environment. We have no science—what they call a predictive ecology methodology.

Even last—as late as last November, the National Science Foundation went in front of a congressional committee and said they were just beginning to develop the first meetings to put together the first studies for such a science of risk assessment.

The Office of Technology Assessment and other agencies of government have acknowledged that they are just beginning to look into the development of such a science of risk assessment.

For the life of me, Congressman, I can't understand how on the one hand, agencies are authorizing releases into the environment, when on the other hand, they acknowledge they have no science yet to judge the risk.

The agencies say they are going case by case. I think that's putting the cart before the horse. Let's see first if we can develop such a science to develop some risk assessment. Let's bring the best scientific expertise in the country together. Let's invite the environmental scientists who have knowledge in this area to be part of the process. That means the entomologists, and the population geneticists, and the microbial ecologists, and the meteorologists—they should all be involved. They are not involved. And they, only begrudgingly, have some Federal agencies begun that process.

But I think we again are repeating the mistakes of petrochemical and nuclear. We are rushing in before there is a science of risk assessment, and before the appropriate regulatory protocols have been put in place to assure public safety and environmental safety.

Mr. VOLKMER. All right.

I recognize the gentleman from Iowa before we have to go vote, if he has any questions.

Mr. BEDELL. Real quickly.

You do not question the efficacy or the proper use of this particular vaccine that we are talking about here, do you, Dr. Rifkin? You don't think it presents any danger, do you?

Mr. RIFKIN. Congressman, I don't know because the appropriate expertise that should have been brought to bear has not looked at whether it is safe or not.

What we have here is the Animal and Plant Health Inspection Service relying on tests that were done by NovaGene and Biologics. But the appropriate experts, which the Recombinant DNA Advisory Committee could have brought together to look at this particular field test and commercial licensing were never consulted.

I see nowhere in the literature—nowhere in the literature—where they invited a panel of environmental scientists to look at this experiment—entomologists, population geneticists, microbial ecologists. In contrast, EPA has both in-house expertise and advisory panels that they bring to bear in looking at the ecological question.

Mr. BEDELL. Oh, I didn't understand this was an issue. So, at least in your opinion, even the tests that were made on the 1,400 hogs do not necessarily prove that this is not—that this is a safe vaccine?

Mr. RIFKIN. That's right. It could prove to be very safe. It could be—it could prove to be a product with immense potential.

Mr. BEDELL. But—

Mr. RIFKIN. But we simply don't know, because I have not seen in the environmental assessment that was issued by the Department of Agriculture any reports by environmental scientists that they brought in on this process to look at all the possible implications of the field test, and to monitor them, and to ask the appropriate questions.

In contrast, now at EPA, those questions are being asked in relation to some other experiments, one of which, as you know, is in your district, Congressman Volkmer.

Mr. BEDELL. Well, I don't know that we can particularly blame Dr. Kit for what he's done, or any of the others. They saw an opportunity the way the law worked to what they could do in order to benefit themselves, and I think that's the way our society works.

I do think, however, Mr. Chairman, that we have a real responsibility to see that things are corrected in such a manner that the public can feel that there's adequate protection of them at the same time we take advantage of the new research as to how it can help people, such as yours and my pork producers, and others.

Mr. RIFKIN. I'd like to say one thing about the initial work on this.

Mr. VOLKMER. Make it short, we have a vote on, go ahead.

Mr. RIFKIN. All right, very quickly. I think that it is yet to be seen whether Dr. Kit and Baylor College of Medicine and Texas A&M were or were not responsible. My own personal belief, after looking at the documents and the material, is that there likely is some culpability here in skirting the regulatory process knowingly. And I think that an investigation by the NIH is called for.

Mr. VOLKMER. All right. Thank you very much.

Mr. RIFKIN. Thank you.

Mr. VOLKMER. And I would like to pursue a few other things but we do have time constraints—we have another vote on. And with that, we will conclude the hearing rather than coming back. And thank you very much for coming, Mr. Rifkin.

[Whereupon, at 2:05 p.m., the joint hearing was adjourned.]

APPENDIX 1

CORNELL UNIVERSITY MEDICAL COLLEGE
DEPARTMENT OF MICROBIOLOGY



1300 YORK AVENUE
NEW YORK, N. Y. 10021
Telephone: (212) 672-6540

29 April 1986

Dr. Eileen Lee
Committee on Science and Technology
U.S. House of Representatives
Washington, D.C.

Dear Eileen,

I shall try to cover both major points you have raised. With regard to the "final" definitions in Attachment C that Dr. Young et al are suggesting, both Monica Riley and I are opposed to excluding from regulatory reviews intergeneric recombinants containing "well-characterized, non-coding regulatory sequences." Such recombinants may well have extended host ranges or altered phenotypes that need to be documented before they could be considered safe for release.

The PRV environmental assessment and finding of no significant impact statement by APHIS, USDA appears sound as far as it goes. It does not state though that the mutant has been sequenced in the critical region or that it does not contain foreign sequences from the plasmid. However, the phenotypic testing for spread, virulence, and stability look pretty good. As I understand the current guidelines of the NIH RAC the mutant strain was exempt from the guidelines until release was considered. All released of of engineered organisms, regardless of final content needs to be reviewed by the RAC. How the USDA fits in with its RAC is unclear to me. Also at which level the first release, even as a test occurred, is of interest. If it was done as part of the Baylor patent it should have probably gone to the NIH RAC first. If the company did it then it's up to the lawyers. I don't think it matters who funded the research if B. Kit did the tests as a Baylor faculty member since the University clearly is a recipient of NIH funds.

With regard to your technical question. When the TK⁻ inoculum was increased 10X to 10^{6.3} TCID₅₀, 2 pigs did have virus in nasal secretions, but even here it was not infectious in whole animals. The isolation appears to be a function of an extremely high intramuscular inoculum. I do not find the data worrisome.

I hope this is helpful.

Sincerely,

A handwritten signature in dark ink, appearing to read "Ken Berns".

Kenneth L. Berns, M.D., Ph.D.
R.A. Rees Fritchett Professor
and Chairman

APPENDIX 2

Report of the Recombinant DNA Subcommittee on the Research Activities of Dr. Saul Kit with regard to the Pseudorabies Project

Beginning in 1981, Dr. Saul Kit and co-workers initiated a series of experiments using recombinant DNA technology to fractionate and clone portions of the viral genome of a herpes virus known as pseudorabies virus. This project appeared to be an extension of ongoing research in the laboratories of Dr. Kit involving herpes viruses and their biochemistry. In those studies, Dr. Kit and co-workers filed under the Guidelines for Research Involving Recombinant DNA Molecules of the National Institutes of Health ("NIH Guidelines") two generic applications (referencing herpes viruses) for approval and one specific application (referencing pseudorabies) for approval from the Subcommittee on Recombinant DNA of Baylor College of Medicine (the "Subcommittee"). Approvals for this research were granted by the Subcommittee. Subsequently, Dr. Kit applied for Baylor College of Medicine Vivarium experimentation involving herpes viruses in mice and rabbits. This was considered independently by the Vivarium Committee and was approved. Annual updates confirmed that his projects were active and unchanged. There were no transmittals of information to the Subcommittee or to the Baylor Biosafety Committee by Dr. Kit regarding experimentation outside the confines of Baylor College of Medicine, nor were there any involving animal inoculations beyond those detailed above for the Baylor Vivarium.

No further detailed reviews of Dr. Kit's activities by the Subcommittee or biosafety Committee were held since his annual renewal statements submitted to the Subcommittee always indicated that no change in research protocols had occurred. However, verbal communications to this subcommittee by telephone from the National Institutes of Health (NIH) and by Dr. James Wild, Chairman of the Texas A & M Biosafety Committee, have prompted two meetings between Dr. Kit and a panel established to investigate this matter and meetings of the Subcommittee itself. The investigative panel consisted of Drs. Savio Wot, Janet Butel and Robert Couch. This panel had two meetings with Dr. Kit. Dr. Stewart McConnell of Texas A & M University accompanied Dr. Kit to the first panel meeting. The Subcommittee has met twice to discuss the findings of the investigative panel and to discuss Dr. Kit's research at Baylor and his involvement in research beyond the confines of Baylor College of Medicine. Having carefully evaluated the documentary evidence, the statements of Dr. Kit as well as the statements and allegations of Dr. McConnell and the information provided by Dr. Wild, the Subcommittee has reached the following conclusions:

1. The pseudorabies virus produced by Dr. Kit for subsequent use in vaccine experimentation involved recombinant DNA technology as defined in the NIH Guidelines.

2. The final product as the vaccine per se must be regarded as a recombinant DNA virus or as containing recombinant DNA molecules.
3. Dr. Kit registered with the Subcommittee his laboratory experimentation which was conducted within the Biochemical Virology laboratories at Baylor College of Medicine and was granted approvals.
4. The pseudorabies field trials conducted at the Maddox farm in central Texas under the auspices of Texas A & M University (TAMU) depended upon the substantial involvement of Dr. Kit as co-principal investigator. Dr. Kit, with the TAMU investigator, co-signed the application in his capacity as a professor at Baylor College of Medicine for permission from the Texas Animal Health Commission to conduct the field trials under the auspices of TAMU. The letter from the Commission granting authority to proceed with the trials was addressed to Dr. Kit also in his capacity as a Baylor faculty member.
5. Dr. Kit provided no disclosure to the Subcommittee of his intention for subsequent experimentation using this recombinant DNA virus vaccine in field trials later performed at or under the auspices of Texas A & M. Disclosures of research by a faculty member beyond that approved by the Subcommittee are required by NIH Guidelines and Baylor policy. Baylor College of Medicine was not notified of nor did it approve such experimentation.
6. The Subcommittee found that the inoculation of greater than 1400 swine at the Maddox farm in central Texas constituted a release into the environment, although the Subcommittee noted that Dr. Kit had relied on a USDA memorandum to the contrary.
7. In view of the NIH Guidelines which prescribe release into the environment of recombinant DNA molecules without prior approval by the Subcommittee and by the NIH, we find no evidence that Dr. Kit complied with this mandated requirement. No notification was provided to the Subcommittee, nor was the Subcommittee consulted regarding this matter.
8. The Subcommittee reaffirms that all research involving recombinant DNA molecules conducted at Baylor College of Medicine or conducted by Baylor faculty members in their official capacity is subject to the NIH Guidelines and Baylor College of Medicine policies.

9. Therefore, the Subcommittee concludes that Dr. Kit, as part of a research team, actively participated in experiments performed outside of Baylor College of Medicine that violated the NIH Guidelines and Baylor College of Medicine policies. In addition, while the degree of cooperation and collaboration with the Texas A & M University system in such experimentation must be recognized, it cannot be interpreted as absolving Dr. Kit as a Baylor faculty member from such a breach of guidelines.
10. The Subcommittee further finds that Dr. Kit should have sought the advice and approval of the Subcommittee in interpreting NIH Guidelines prior to the initiation of the field tests.
11. The Subcommittee also concludes that the multiplicity of federal guidelines from different federal agencies - in this case the NIH and the Department of Agriculture - may have produced confusion amongst the investigators and possibly compounded the potential for a breach of the guidelines of one federal agency.

May 2, 1986

APPENDIX 3

THE TEXAS AGRICULTURAL EXPERIMENT STATION
THE TEXAS A&M UNIVERSITY SYSTEM
College Station, Texas 77843-2147

Office of the Director

(409) 845-8484



April 28, 1986

Ms. Eileen Lee
Committee of Science and Technology
822 House Annex 1
300 New Jersey Avenue SE
Washington, D. C. 20515

Dear Ms. Lee:

Enclosed you will find a copy of a letter addressed to Dr. William Gartland, Director of the Office of Recombinant DNA Activities in the National Institutes of Health, dealing with a recent assessment of the situation at Texas A&M as regards the pseudorabies vaccine studies.

Sincerely,

Neville P. Clarke
Director

NPC/k1b

Enclosure

TEXAS A&M UNIVERSITY
 BIOCHEMISTRY AND PHYSICS
 COLLEGE STATION, TEXAS 77843
 PHONE 409/845-1111 3-2728

April 25, 1986



Dr. William Gartland, Jr.
 Director, Office of Recombinant DNA Activities
 National Institute of General Medical Sciences
 Rm. 3810, BLDG 31
 9000 Rockville Pike
 Bethesda, Maryland 20205

Dear Dr. Gartland:

This letter presents a report of a potential violation of the procedures established in "Guidelines for Research Involving Recombinant DNA Molecules" (November 23, 1984). On behalf of Texas A&M University System, I present this report according to the Guidelines (Section IV-B-3-g).

As the result of the notoriety of the USDA licensing of a modified-live pseudorabies virus as a veterinary vaccine against Aujeszky's disease in swine, one of the principal investigators notified our IEC of potential concerns regarding those studies. Dr. Stewart McConnell, Professor Emeritus, Department of Veterinary Microbiology and Parasitology, submitted several published reports of the development and use of the vaccine. Subsequently, the TAMU System IEC evaluated those studies and has come to the following conclusions.

An attenuated deletion mutant, modified-live pseudorabies viral vaccine developed through recombinant DNA technologies was utilized to immunize a quarantined swine herd infected with Aujeszky's disease on the Wallace and Tommy Maddox farm in West Central Texas. These studies are reported in an article entitled "Immunization of pregnant pigs in a quarantined swine herd with a thymidine kinase deletion mutant of pseudorabies virus", S. Kit, M. Kit, B. Lemhorn and S. McConnell in "High Technology Route to Viral Vaccines," 1985, American Society of Microbiology, Eds. G.R. Dreesman, J.G. Bronson and R.C. Kennedy, pages 82-99. A new experimental vaccine (patent strain PRV (BUD13) developed by deleting a specific segment of the thymidine kinase gene of an established vaccine strain (Bucharest BUK) was utilized in these studies. This deletion strain was formed with recombinant DNA technologies under P1 containment by Dr. Saul Kit at Baylor College of Medicine and is described in the enclosed article entitled "Attenuated properties of thymidine kinase-negative deletion mutant of pseudorabies virus," Saul Kit, Malon Kit and Eugene C. Pirtle, 1985, Amer. J. Vet. Research 46: 1359-1367.

This immunization and the clinical safety studies preceding it were not presented for evaluation to the Institutional Biosafety Committee at Texas A&M University even though some of these studies involved the participation of members of the Texas A&M University System, its facilities and might have resulted in release of modified-live virus into the environment.

The staff members of the Texas A&M University System involved in these

College of Agriculture

Texas Agricultural Experiment Station

studies were Dr. Stewart McConnell, Dr. D. Bruce Lawhorn (a veterinarian with the Texas Agricultural Extension Service, College Station) and Dr. Charles Livingston (a veterinarian with the Texas Agricultural Experiment Station, San Angelo). These investigators believed that these studies were exempt from consideration under the Guidelines. This belief was based on an interpretation of Section IV-B-C-1 which states that the PI shall: "Make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines" and Section IV-B-5-c which requires that the PI shall: "Submit the initial research protocol if covered under Guidelines Sections III-A, III-B, or III-C (...) to be <sic, "the"> IEC for review and approval or disapproval." Dr. Kit then interpreted Section III-D-2 to exempt the recombinant viral vaccine from the Guidelines and thus no registration with the IEC was necessary. Section III-D-2 exempts "Those <recombinant DNA molecules> that consist entirely of DNA segments from a single nonchromosomal or viral DNA source though one or more of those segments may be a synthetic equivalent."

The TAMU System IEC disagrees with this interpretation and believes that this research is not exempt as described above. The dominant Guideline is presented in the introduction to Section III which defines four classes of recombinant DNA experiments. Section III-A identifies "Experiments which require specific PAC review and NIH and IEC approval before initiation of the experiment." Included in these experiments and defined in Section III-A-2 are those experiments which result in "Deliberate release into the environment of any organism containing recombinant DNA, except certain plants as described in Appendix L." The Guidelines state, in bold type, the following declaration: "IF AN EXPERIMENT FALLS INTO BOTH CLASS III-A AND ONE OF THE OTHER CLASSES, THE RULES PERTAINING TO CLASS III-A MUST BE FOLLOWED." It is the belief of the IEC that the determination of deliberate release is not the option of the PI and any "potential" release must be evaluated by the IEC, NIH and PAC reviews.

The investigators have responded by reference to the USDA Veterinary Safety Memorandum No. 800.68, Section III, "Environmental Considerations", which states: "Under the present NIH guidelines, the deliberate release of any organism containing recombinant DNA into the environment is subject to review and approval by appropriate Federal agencies. In normal husbandry and laboratory practices, veterinary biological products are not considered to be released into the environment. In the event that a veterinary biological product would be considered to be released into the environment, the issuance of a license or import permit may require an environmental impact statement and interagency approval." In the broad sense of interpretation, this perspective would permit the use of any reconstructed vaccine without evaluation following an "informed determination" that no viral shedding was observed from infected live animals.

The IEC notes that this memorandum was dated after these studies were performed. But more importantly, this memorandum has been interpreted by the PIs involved in the pseudorabies studies to prescribe a very important exclusory clause to the NIH Guidelines. The TAMU System IEC believes that this memorandum should not pre-empt the Guidelines and such experimentation should be presented for IEC, NIH and PAC reviews.

I would like to ask your interpretation of our findings.

Although the defined procedures required for the evaluation of these studies were not followed, the vaccine does appear to have been an effective immunologic agent with no adverse consequences. As the result of this situation, our IBC and the administration have undertaken new efforts to insure that all components of our University System (the University, the Agricultural Experiment Station, and the Agricultural Extension Service including off-campus programs) are aware of their responsibilities. Another problem involved in this situation is inherent in cooperative research endeavors such as this one between Baylor College of Medicine and various components of the TAMU System. Rhetorically stated: Who is responsible for what aspects of the research program? We will make certain that PIs are aware that each institution involved in their collaborative programs must be involved in the biosafety decisions.

Thank you for your continuing efforts in behalf of our scientific community.

Please contact me directly if you need any additional information.

Sincerely,

James R. Wild
James R. Wild
Chairman, IBC
(409) 845-4943

xc: Dr. N.P. Clarke, Director TAES
Dr. G.P. Eaton, Provost, TAMU
Dr. J.P. Jordan, USDA-ARDC, Washington DC
Dr. A.J. Garber, IBC Chair, Baylor, Houston
Dr. S. Kit, Baylor College of Medicine, Houston
Dr. S. McConnell, TAMU
Dr. D.B. Lawhorn, TAEX, College Station
Dr. C. Livingston, TAES, San Angelo

APPENDIX 4



DEPARTMENT OF AGRICULTURE
OFFICE OF THE SECRETARY
WASHINGTON, D. C. 20250

June 27, 1986

Honorable Harold L. Volkmer
Chairman, Subcommittee on Investigations
and Oversight
Committee on Science and Technology
U.S. House of Representatives
Washington, DC 20515

Dear Mr. Chairman:

This is in reference to your letter of June 16, 1986, asking for our response by June 30, 1986, to a number of questions concerning the April 29, 1986, hearing on the licensing of TechAmerica's pseudorabies vaccine. As requested, attached is the chronology of events concerning the licensure of the vaccine. The chronology and most of the information you requested are contained in the material that was provided prior to the hearing. However, we will be pleased to provide you with the information in the format you requested.

Staff members are currently in the process of preparing answers to your questions.

Since we wish to provide you with as complete a response as possible to this very important issue, it will be difficult, if not impossible, to respond accurately by June 30. Therefore, we respectfully request that our response date be extended to July 8, 1986.

Sincerely,

Karen K. Darling
Acting Assistant Secretary
Marketing and Inspection Services

Enclosure

CHRONOLOGY

Licensure of Genetically Engineered Thymidine Kinase Negative (TK⁻)
Pseudorabies Vaccine, Modified Live VirusTechAmerica Group, Inc.
Omaha, NE 68134

United States Veterinary Biologics Establishment License No. 272

December 27, 1984

Received:

1. A license application (VS Form 14-3).
2. A protocol designed to demonstrate that upon passage in host animals, or vaccine recipients, the avirulent properties of the virus are stable and will not revert to pathogenicity. The study was to be conducted at Michigan State University.
3. Two literature references:
 - a. Describing the shedding pattern in swine of virulent and attenuated pseudorabies virus (Am. J. Vet. Res., Nov. 1983).
 - b. A preprint of a paper "Attenuated Properties of Thymidine Kinase Negative Deletion Mutant of Pseudorabies Virus." (Article to be submitted to Am. J. Vet. Res.)

(NOTE: Although we lacked specific details concerning each step used to develop the avirulent TK⁻ seed virus:

- (1) It was unquestionably clear that we were dealing with a genetically engineered virus or one that had been modified by recombinant DNA procedures.
- (2) Undoubtedly, this specific information could have been obtained prior to issuance of the patent, but it was not required at this time.
- (3) The initial phases of the licensing process could proceed without these details.

March 22, 1985

Received:

1. Proposed efficacy and field trial evaluation protocol was submitted for evaluation. Requested that the efficacy trial be conducted by Dr. B. Thacker (Michigan State University, Department of Large Animal Clinical Services, College of Veterinary Medicine, East Lansing, Michigan) under the auspices of Biologics Corporation.
2. Movement of unlicensed product for evaluation and/or research purposes; done in accordance with Title 9, Code of Federal Regulations (9 CFR), Part 103.3. This documentation included permission for the test by appropriate State officials from the Michigan Department of Agriculture.
3. Reply to letter of March 22, 1985 requesting authorization for proposed studies was sent on April 15, 1985.

July 1, 1985

Received:

Data concerning TechAmerica's production and testing of the Master Seed Virus (MSV) for this product. (Master Seed is the virus at a specified passage level which has been selected and permanently stored by the producer and from which all other seed passages are derived within permitted levels (9 CFR 101.7(a)).

August 2, 1985

Replied:

Provided more information, such as passage details prior to authorization for submission of samples to National Veterinary Services Laboratories (NVSL) for confirmatory testing.

August 16, 1985

Initial efficacy data reviewed. TechAmerica Group, Inc., was informed that additional information was required.

(NOTE: Additional efficacy data accepted November 4, 1985.)

September 5, 1985

TechAmerica's completed Master Seed Virus testing data was submitted to APHIS.

September 6, 1985

Request to NVSL to initiate testing of Master Seed Virus. Telephone authorization to TechAmerica Group, Inc., to send samples to Ames, Iowa.

September 28, 1985

TechAmerica Group, Inc., requests permission for field trials in Minnesota.

October 1, 1985

Copy of Dr. S. Kit's patent received.

October 10, 1985

Test results from NVSL show that Master Seed Virus is satisfactory. Virus can now be used to prepare prelicensing serials in accordance with the approved Outline of Production and other prelicensing usages.

October 14, 1985

TechAmerica Group, Inc., requests permission for field trials in Illinois.

October 17, 1985

Report on Antigen Extinction Test to determine effective titer for immunogenicity testing.

October 22, 1985

Repeat Immunogenicity Test Data submitted.

VS Form 14-8 on each of the three prelicensing serials. (This form contains the results of all Outline of Production, Section V tests for serial release.)

Vaccine Stability Data submitted.

October 28, 1985

The preliminary backpassage results, which strongly indicated no reversion or transmissibility were discussed in a telephone conversation on October 28, 1985, prior to submission of completed test results on November 27, 1985.

Since this information was consistent with all previous data, telephone authorization was given on October 28, 1985, to conduct field safety tests in Minnesota and Illinois.

November 4, 1985

Additional efficacy data accepted.

November 5, 1985

TechAmerica Group, Inc., requests permission to field test in Iowa. (Written authorization given November 29, 1985.)

November 14, 1985

Additional safety testing in mice and sheep submitted.

November 27, 1985

Backpassage report.

Field safety testing results.

Passage history of seed virus from Dr. S. Kit.

December 3, 1985

Seed virus test results from NVSL - Satisfactory.

(Determined TK activity and gel patterns showing gene deletion.)

December 12, 1985

Test results from NVSL on three prelicensing serials - satisfactory.

December 26, 1985

Amended Outline of Production.

January 16, 1986

License issued.

APPENDIX 5



United States
Department of
Agriculture

Animal and
Plant Health
Inspection Service

Washington, DC
20250

July 16, 1986

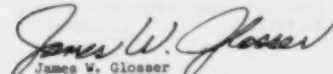
Dr. Irene Glowinski
Subcommittee on Investigations
and Oversight
Committee on Science and Technology
House of Representatives
Washington, D.C. 20515

Dear Dr. Glowinski:

Pursuant to your request at the biotechnology briefing yesterday, enclosed is an advance copy of the final response to the 19 questions contained in Congressman Volkmer's June 16, 1986, letter.

These responses were prepared and cleared through this Agency and the Office of General Counsel on July 7. However, it is our understanding that these responses are being cleared by other agencies in the Department. We apologize for the delay in this process.

Sincerely,


James W. Glosser
Associate Administrator

Enclosure



DEPARTMENT OF AGRICULTURE
OFFICE OF THE SECRETARY
WASHINGTON, D. C. 20250

Honorable Harold L. Volkmer
Chairman, Subcommittee on Investigations
and Oversight
Committee on Science and Technology
House of Representatives
Washington, D.C. 20515

Dear Mr. Chairman:

Pursuant to your request dated June 16, 1986, we are submitting the following information in answer to your questions regarding USDA's licensing of TechAmerica's pseudorabies vaccine and other related issues. The chronology which you requested was submitted on June 27, 1986.

Sincerely,

Enclosure

RESPONSES TO QUESTIONS

- 1Q. When TechAmerica Group submitted its application to USDA in December 1984, did the application contain all of the relevant data necessary for the agency to reach a decision in this case?
- Did the submission contain the "outline of production" detailing how the vaccine would be made?
- 1A. All of the relevant data necessary to reach a decision with respect to licensing a product is not normally submitted with a license application. When TechAmerica Group submitted its application to USDA in December 1984, it did not contain all the relevant data necessary to reach a decision regarding licensing of the company's product. The application was accompanied by an outline of production detailing how the vaccine would be made. There were numerous additional steps that had to be completed during the licensing process, including the submission of additional documentation in support of the application. TechAmerica was required to follow all of the prescribed licensing procedures and to demonstrate that the vaccine was pure, safe, potent, and efficacious before a decision was reached regarding its product.
- 2Q. Why was the vaccine not classified as a recombinant organism when TechAmerica Group originally filed its application in December of 1984?
- 2A. Materials originally submitted with TechAmerica's license application clearly indicated that the company's product was recombinant derived. All animal biologics which are being reviewed for licensing or which are licensed by APHIS are assigned a code number for internal use by the agency. Recombinant derived products receive an "R" code number to distinguish them from other products. At the time of TechAmerica's application the "R" code system had not yet been developed.
- 3Q. Did the fact that Dr. Kit's application to patent the vaccine was pending when TechAmerica filed its license application interfere with APHIS' ability to review confidential business information related to the application?
- 3A. The fact that Dr. Kit had a patent pending for his vaccine virus did not interfere with APHIS' ability to review confidential business information related to the application. Before making the licensing decision, the Agency received and reviewed all the

information necessary to determine that the vaccine was pure, safe, potent and efficacious.

4Q. What new information provoked the reclassification of OMNIVAC as a recombinant organism?

- If none, then why did the agency change the classification?

4A. There was no actual "reclassification" of the recombinant derived OMNIVAC vaccine. Once the new recombinant product code numbering system was in place it was determined that both recombinant and recombinant derived products would be assigned recombinant ("R") numbers. The Agency simply applied the new system to OMNIVAC which had received its original code number before the new system was developed.

5Q. Why was the final classification of the vaccine decided prior to USDA authorizing the field tests of the vaccine at several state experimental stations?

- What was the purpose of reclassifying the vaccine after the field test and prior to the licensing?

5A. When the product license application was initially received, the "R" numbering system had not yet been established, so the product was assigned a regular code number. The "R" numbering system came into use in March of 1985. When the firm became involved in field testing and was closer to having the product licensed, the file again came under active review and the code number was changed. OMNIVAC was assigned an "R" code number in the Fall of 1985 to conform to the new veterinary biologics coding system. The coding system is used to designate or code each license or outline of production. It is an internal, administrative alphanumeric system which denotes product type, agents used, viability of the vaccine, the substrate, and other miscellaneous variables.

6Q. If veterinary services had classified the vaccine as a recombinant organism prior to field testing, would the Agricultural Recombinant DNA Review Committee (ARRC) have been involved in the review of the application?

6A. The code revision was not relevant to the question of ARRC review.

- 7Q. Does any division outside of Marketing and Inspection review an application to license a product?
- 7A. Normally, no other agency outside APHIS reviews license applications for animal biologics. However, there are times when other organizations are consulted regarding licensing actions. For example, it is not uncommon to consult with the Food Safety Inspection Service and the Agricultural Research Service of USDA. Outside organizations such as the Food and Drug Administration and the Communicable Disease Center may also be consulted.
- 8Q. At the time that TechAmerica's application was being reviewed, what were the rules regarding ARRC involvement in review of recombinant DNA derived veterinary biological products?
- How was the ARRC notified of a submission to the agency which required ARRC review?
 - How was that done in the OMNIVAC case?
 - What other review groups in or out of USDA were utilized in the review of the TechAmerica application to license OMNIVAC?
- 8A. At the time that TechAmerica's license application was being reviewed there were no rules regarding ARRC involvement in the review of recombinant derived veterinary biologics. APHIS is in the process of revising and updating its procedures for reviewing recombinant products.
- There were groups and individuals, other than the APHIS Veterinary Services Biologics Staff, who were involved in the review of OMNIVAC. Some of these were the National Veterinary Services Laboratories in Ames, Iowa, a biometrician from the APHIS Technical Assessments Staff, and a researcher from the National Animal Disease Center in Ames, Iowa.
- 9Q. What agency regulations or practices ensure that ARRC review is part of the process for licensing genetically-engineered veterinary drugs and biologicals?
- 9A. There are no regulations or practices providing for ARRC review of animal biologics license applications. However, APHIS has established a Veterinary Services Biotechnology Committee, which includes both inter-agency and intra-agency representation, and which functions as a coordinating committee to review licensing policy and procedure (including NEPA considerations).

10Q. In Mr. Tracy's testimony from the hearing, he stated that USDA is not required to publish an environmental assessment prior to licensing and releasing a genetically-engineered veterinary drug or biological. Please explain.

- Under what conditions are you required to publish an environmental assessment? Who is required to prepare and publish it?
- Why was the environmental assessment document not properly prepared at the time of license approval of the vaccine?

10A. When an Agency conducts an environmental assessment the regulations of the Council on Environmental Quality (CEQ) implementing the National Environmental Policy Act (NEPA) do not require publication of the environmental assessment. Similarly, the Department's regulations and APHIS' guidelines implementing NEPA do not require publication of the environmental assessment. NEPA requires federal agencies to prepare an Environmental Impact Statement (EIS) for an agency action that significantly affects the quality of the human environment. APHIS conducts environmental assessments to determine the likely environmental impacts of a proposed agency action. If it is significant, APHIS prepares an EIS, if it is not, the agency issues a Finding of No Significant Impact (FONSI).

APHIS did not consider licensing OMNIVAC as an agency action of significant environmental impact because of the vaccine's safety and the virus' inability to replicate outside the animal. Therefore, an environmental assessment document was not prepared prior to licensing. However, the procedures followed by APHIS in licensing OMNIVAC were scientifically sound and required the submission of data sufficient to fully assess environmental concerns. The environmental assessment relevant to the licensing of OMNIVAC was separately documented after licensing to fully reflect the measures and procedures taken during the licensing process to assure that environmental concerns were addressed, and a finding of No Significant Impact was published.

11Q. During the recent GAO investigation of USDA's Biotechnology effort, Dr. Shibley listed for GAO a number of procedures that would be followed prior to licensing a genetically-engineered veterinary biological. These included: looking closely at the NIH guidelines; conferring with the "legal people" in USDA; designing an acceptable procedure complete with risk analysis for the specific case; review by the APHIS Biotechnology Work Group; consultation between the ARRC and NIH; and finally, publication of

the overall request and review process in the Federal Register for comment. Why were those procedures not followed in this case?

- 11A. During the licensing of OMNIVAC, TechAmerica was required to strictly follow all applicable regulations and standards in place at the time and to demonstrate that its product was pure, safe, potent, and efficacious. Some of the procedures discussed by Dr. Shibley were not part of APHIS' licensing requirements and were simply raised as points of discussion in the agency's effort to revise and update its review process.
- 12Q. Who in USDA would decide whether additional tests are necessary prior to licensing a recombinant DNA derived veterinary biological product?
- What significant additional tests were required after USDA classified the vaccine as a recombinant organism?
- 12A. Normally, the product reviewer and the Senior Staff Veterinarian, in conjunction with the National Veterinary Services Laboratory, would decide whether additional tests are necessary prior to licensing a product. APHIS was aware of the fact that OMNIVAC was a recombinant derived product at the time of the original application. Therefore, the change in the product code number had no bearing on test requirements. However, it should be noted that since this was a recombinant derived product new test procedures were initiated to characterize the virus, including thymidine kinase assays and restriction endonuclease characterization of the master seed virus.
- 13Q. A letter from the National Veterinary Services Laboratory to Dr. Shibley, indicated that laboratory tests to confirm the purity and identity of the pseudorabies vaccine were not completed until February 7, 1986. The license for OMNIVAC was issued on January 16, 1986. Why was the OMNIVAC vaccine given a license for marketing prior to completion of these tests as required by APHIS?
- 13A. All of the tests required for licensing were completed prior to issuance of the license. The tests referred to in this question provided supplementary, additional data and were not necessary for licensing.
- 14Q. Who in USDA determines whether an applicant for a veterinary biological product license has complied with the applicable NIH guidelines?
- How is this determination made?

- 14A. The product reviewer as well as other members of the biologics licensing staff would determine whether applicants have complied with applicable guidelines, or portions thereof. Compliance with NIH guidelines is voluntary in the case of commercial, non-federally funded establishments.
- 15Q. The tests conducted at the National Animal Disease Laboratory in Ames, Iowa involved animals in confinement under class 3 containment conditions. What does this mean?
- If it was necessary to isolate the animals for these series of laboratory tests, why did USDA not suggest similar guidelines for the field test in the various states?
- 15A. Class 3 containment consists of a high degree of isolation precluding release of materials into the environment. Such containment was not required during field tests because the vaccine had been shown to be safe and posed no threat to the environment. Containment is advised when determining the safety of a product.
- 16Q. During the hearing, Fr. Bentley testified that he would recommend that the Secretary of Agriculture replace the current ARRC with a committee closely patterned after the existing NIH RAC. Has this recommendation been made?
- How would this change the duties and responsibilities of ARRC?
 - Would this allow the ARRC to use outside reviewers and to review confidential business information as does the NIH RAC?
- 16A. It has been recommended that the ARRC be replaced by the Agricultural Biotechnology Recombinant DNA Advisory Committee (ABRAC) with expanded functions. This committee, which would be composed of public and private sector members, would be a Federal Advisory Committee which would advise the Secretary on scientific and technological issues relating to biotechnology research, and as called upon, the scientific aspects of regulatory functions. Use of outside reviewers and review of confidential business information would be allowed. ABRAC review would be conducted in a manner that avoids conflict of interest while assuring protection of confidential business information.
- 17Q. During the hearing, Mr. Tracy testified that any genetically modified product would first be looked at by an IBC, which would then, under the new guidelines notify USDA of its actions. Who

within USDA will be notified, of what will they be notified, and what actions can an IBC take to approve release of a genetically-engineered product?

- 17A. APHIS-Veterinary Services would be notified of the makeup of a firm's IBC membership in the case of products regulated by APHIS. The committee would review each of the firm's proposals involving products derived from the new biotechnology. The results of the IBC review would be submitted to APHIS. The IBC would not have the authority to approve release.
- 18Q. Has USDA published the implementing regulations to require permission for the intra-state shipment of viruses for field testing, as noted in the 1985 Farm Bill? If not, when are they expected to be published?
- 18A. Proposed amendments to the veterinary biologics regulations dealing with intrastate shipment of products for research are now in draft form and should be ready for publication very soon.
- 19Q. What statutory authority does USDA have to require an experimental use permit prior to the release of a genetically-engineered organisms into the environment?
- If a plant product is not a plant pest, what statutory authority does USDA have to require a permit prior to release into the environment?
- 19A. The Department has extensive authorities upon which it can rely in dealing with genetically engineered organisms. These include: the Food Security Act of 1985; the Virus-Serum-Toxin Act; the Federal Plant Pest Act; the Plant Quarantine Act; the animal quarantine laws; the Federal Meat Inspection Act; and the Poultry Products Inspection Act. Regulations under the Virus-Serum-Toxin Act specifically address the issue of preparation, distribution and evaluation of experimental animal biologics (9 C.F.R. Part 103). Part 122 of Title 9 of the Code of Federal Regulations also contains regulations promulgated pursuant to the Virus-Serum-Toxin Act and the Animal Quarantine laws which control the shipment of organisms and vectors. The Federal plant pest regulations require a permit prior to the importation or interstate movement of plant pests (9 C.F.R. Part 330).

If the Department determines that a plant product is not a plant pest but is a beneficial plant product, then the Department does not construe the Federal Plant Pest Act or the Plant Quarantine Act as granting authority to require a permit prior to release of

the beneficial plant product into the environment. The proposed regulations under the Federal Plant Pest Act and the Plant Quarantine Act are structured to prevent the release into the environment of genetically engineered organisms which are plant pests or which there is reason to believe are plant pests.

APPENDIX 6

TechAmerica Group, Inc.

Biologics Corporation
(Division of TechAmerica Group, Inc., Animal Health Division)
2720 N. 84th St./P.O. Box 34325/Omaha, NE 68134
(402) 393-7440

July 16, 1986

Mr. Greg Simon
Subcommittee on Investigations
and Oversight
822 House Annex #1
Washington, D.C. 20515-6307

Dear Mr. Simon:

The following are responses to the questions in Mr. Volkmer's letter of June 16, 1986 (see attached copy).

1. Yes. All information provided by Dr. Kit was supplied to the U.S.D.A. This included all the information available to us at the time describing the vaccine virus and it's characteristics.
2. No. We provided the U.S.D.A. with a full disclosure of information available to us. The decision to classify the organism as a recombinant was made the following November.
3. At the time, we were well into the licensing process and proceeding with the rigorous U.S.D.A requirements. Consequently, we viewed it only as a change in our product code number from 1891.20 to 1891.RD.
4. No.
5. We reviewed the tests, protocols and virus prior to all testing. The consensus was that this vaccine presented no unusual risk when compared to the current vaccines or the field virus circulating in nature.

Mr. Greg Simon
July 16, 1986
Page 2

6. No. Discussions were always under U.S.D.A. jurisdiction and all testing conducted with their prior approval. Specific areas of the NIH Guidelines exempted us from review, however, we were always under the review of the A.P.H.I.S. - U.S.D.A. system.

The NIH Guidelines were designed to provide a regulatory framework for those products not subject to any pre-market approval statutory requirements. We were always under, during this time, the U.S.D.A. jurisdiction through the Virus-Serum-Toxin Act.

I hope the responses are sufficient for the purpose(s) intended. Should you require additional information, please feel free to call me.

Sincerely,

Michael J. Bartoski, Jr.

Michael J. Bartoski, Jr., Ph.D.
Vice President of Operations

MTB/krk

Attachment

SEN. POLAR, Maine, Chairman

ROBERT A. COLE, Jr., Idaho
 ROBERT E. BROWN, Jr., California
 JAMES H. SCHWARTZ, New York
 MARVIN L. LEVY, Tennessee
 THOMAS E. BERRY, Colorado
 EDWARD VANDERBILT, Pennsylvania
 DAN GLENN, Kansas
 ROBERT A. YOUNG, Missouri
 HENRIETTA L. VOLKMER, Missouri
 BILL ROSEN, Florida
 STEVE LINDBERG, New York
 RALPH W. HALL, Texas
 DAVID BUCKLEY, Connecticut
 DONALD T. BENTLEY, California
 MICHAEL A. GARDNER, Texas
 THE VANDERBILT, North Carolina
 JOSEPH W. BELL, Nevada
 ROBERT E. TORRICELLI, New Jersey
 FREDERICK C. SCHLESER, Virginia
 TERRY BRUCE, Illinois
 RICHARD W. STALLINGS, Idaho
 BART TROMBON, Tennessee
 JAMES A. TRAPNICK, Jr., Ohio

U.S. HOUSE OF REPRESENTATIVES
 COMMITTEE ON SCIENCE AND TECHNOLOGY

SUITE 2321 RAYBURN HOUSE OFFICE BUILDING
 WASHINGTON, DC 20515
 (202) 225-8371

June 16, 1986

CHARLES L. LARSEN, Jr., New Mexico
 ROBERT E. WALLACE, Tennessee
 JAMES MONROE, New York
 CLAUDE SCHWARTZ, Rhode Island
 BERNARD L. BOWEN, New York
 TOM LLOYD, Florida
 DON RITTS, Pennsylvania
 JOE W. BOWEN, Connecticut
 BOB PACKARD, California
 JOE BERRY, Kansas
 ROBERT C. SMITH, New Hampshire
 PAUL D. HART, Michigan
 WILLIAM W. CORRY, Jr., North Carolina
 JOE BARTON, Texas
 B. FRANK SLAUGHTER, Jr., Virginia
 DAVID S. BROWN, Utah

DANIEL P. HANSEN
 Executive Director
 ROBERT C. KETCHUM
 General Counsel
 JUDY GROSS VERMILION
 Reception Staff Director

Michael J. Bartkoski, Jr., Ph.D.
 Vice-President of Operations
 Biologics Corporation
 TechAmerica Group, Inc.
 15th and Oak
 Elwood, KS 66024

Dear Dr. Bartkoski:

Thank you for your time and effort with respect to the April 29, 1986 hearing on the licensing of a pseudorabies vaccine. To complete the hearing record, I would appreciate your written responses to the following questions:

1. Dr. Kit testified that in November 1984, he gave TechAmerica all of the published information regarding the nature of the OMNIVAC vaccine, including a description of how the genetic material had been deleted. In December 1984, TechAmerica applied to USDA for a license to market this vaccine. Did TechAmerica's application to USDA contain all of the information submitted to TechAmerica by Dr. Kit that would enable USDA to determine whether or not the vaccine should be classified as a recombinant organism?
2. At the time USDA reviewed TechAmerica's application, were there any conversations between TechAmerica and officials of the Veterinary Services division regarding whether the vaccine should be classified as a recombinant organism?
 - If so, why was the organism not classified as "recombinant" at that time?
3. Did TechAmerica consider the reclassification of OMNIVAC as a recombinant organism to require merely a change of label or was TechAmerica aware that reclassification could trigger certain pre-licensing procedural requirements?

TechAmerica Group, Inc.

June 16, 1986

Page Two

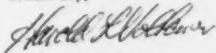
4. In June 1984, Drs. Kit, Leshorn, and McConnell conducted a field test on the Maddox Herd of 1,400 pigs in West Central Texas, which provided data on the safety of the vaccine for newborn pigs, pregnant pigs, and young pigs. This experiment indicated that the pseudorabies vaccine was effective in creating a barrier against the spread of the pseudorabies from feral pigs to pigs in the herd.
 - Did TechAmerica know that the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH) had not been apprised of this field test?
5. In 1985 TechAmerica wrote letters to Illinois and Michigan requesting permission to test its vaccine in those states. The letter stated that the tests were intended to measure the safety of a "gene-deleted" vaccine and that the vaccine virus would be "minimally excreted."
 - Did TechAmerica consult with its Institutional Biosafety Committee concerning the possible release of the virus during these tests?
6. Section 3A of the NIH guidelines states that tests of genetically-engineered organisms performed outside of a laboratory require NIH-RAC approval. USDA published a notice in the Federal Register in December 1984 which stated that the agency required its license applicants to comply with NIH-RAC guidelines. Did TechAmerica ever discuss with USDA whether TechAmerica was required to notify the NIH-RAC of its field tests of the vaccine?

Your responses to the above questions should be submitted by June 30, 1986 to:

Mr. Greg Simon
Subcommittee on Investigations and Oversight
822 House Annex #1
Washington, D.C. 20515-6307

I wish to extend my thanks for your service to the Subcommittee.

Sincerely,



Harold L. Volkmier
Chairman
Subcommittee on Investigations
and Oversight

V:Gg

APPENDIX 7

Not For Sale
Use Only - Not For Sale

Pseudorabies Vaccine
Modified Live Virus
"Notice! For Experimental
Use Only - Not For Sale"

Pseudorabies Vaccine
Modified Live Virus
"Notice! For Experimental
Use Only - Not For Sale"

Label Sketch for Experimental Vaccine

Pseudorabies Vaccine
Modified Live Virus
"Notice! For Experimental
Use Only - Not For Sale"

APPENDIX 8

UNITED STATES DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
 NATIONAL CENTER FOR HUMAN GENE THERAPY
 10-17-85

1. NAME & MAILING ADDRESS OF LICENSEE OR PERMITTEE (PRINTED OR TYPED)
 BIOLOGICS CORPORATION
 DIVISION OF TECHNOLOGICA
 200 NORTH 84TH STREET
 CHICAGO, IL 60634

2. EXPIRATION DATE
 12-31-85

3. PRODUCT IDENTIFICATION NO.
 101-100

4. PRELIMINARY SUBMISSION NO.
 101-100

5. TRUE NAME OF PRODUCT
 PSEUDORABIES VIRUS VACCINE - MODIFIED LIVE VIRUS

6. TEST DATA (10-17-85) (10-17-85) (10-17-85)

TEST REFERENCE	TEST DATES		RESULTS	INSERT CODE	
	STARTED	CONCLUDED		S SATISFACTORY U UNSATISFACTORY I INCONCLUSIVE NT NO TEST	U UNSATISFACTORY I INCONCLUSIVE NT NO TEST
113.27	09-20-85	10-04-85	110/10 No Growth (20°C)		
113.27	09-20-85	10-16-85	110/10 No Growth (25°C)		
113.27	09-20-85	10-16-85	Final Container Samples Free of Mycoplasma		
113.41	10-01-85	10-22-85	2 / 2 Spine Well		
Production Outline V.C.2	09-27-85	10-02-85	PER		
113.135	09-27-85	10-02-85	(Identical)		
113.26	09-24-85	09-26-85	Meisum		
Production Outline V.E.2	11-12-85	11-23-85	Thymidine Kinase Phenotype status: Negative		

7. INVENTORY FOR RELEASE (10-17-85) (10-17-85) (10-17-85)

NO OF CONTAINERS	CONTAINER SIZE (DOSES OR UNITS)	TOTAL QUANTITY (DOSES OR UNITS)	TOTAL NO DOSES	REMARKS
				PRELICENSING SERIAL
				Amended U.S. Form 14-6 showing test results for Thymidine Kinase (TK) Phenotype status
				INDEXES - TO BE REPROCESSED AND RETESTED

8. SIGNATURE (10-17-85) (10-17-85) (10-17-85)

9. DATE DIRECTOR OF REGULATORY AFFAIRS (QUALITY CONTROL) (10-17-85)

10. DISPOSITION BY ARMS (10-17-85) (10-17-85) (10-17-85)

11. TESTS COMPLETED (10-17-85) (10-17-85) (10-17-85)

12. SIGNATURE (10-17-85) (10-17-85) (10-17-85)

APPENDIX 9

April 15, 1985

Mr. T. C. Bevard
Biologics Corporation
2720 North 84th Street
Omaha, NE 68134

Dear Mr. Bevard:

This is to acknowledge receipt of your submission of March 22, 1985, with information to support your license application for Pseudorabies Vaccine, Modified Live Virus, Code 1801.20.

We have reviewed and filed as satisfactory the proposed field study entitled "Efficacy and Safety Evaluation of a Modified Live Pseudorabies Virus Vaccine."

This is your authorization to conduct these trials according to your proposed protocol in Michigan where written permission from State regulatory authorities has been filed with this office. This authorization is valid for 1 year from the date of issuance. A summary of the results must be filed with this office.

The name and address of Biologics Corporation without the United States Veterinary Biologics Establishment License Number must also appear on the experimental label.

Results for standard reference preparations shall be made to National Veterinary Services Laboratories in accordance with Veterinary Services Memorandum No. 700.3 (copy enclosed).

Sincerely,

G. P. Shibley

George P. Shibley, Ph.D.
Chief Staff Microbiologist
Veterinary Biologics Staff
Veterinary Services

Enclosure

CC:
NVSL, Ames, IA (w/cv incom)
VDPC, VS, Ames, IA (w/cv incom)

APHIS:VS:GPSHibley:ext.68674:4-15-85:nom

TechAmerica Group, Inc.

Biologics Corporation
Division of TechAmerica Group, Inc., Animal Health Division
2720 N. 84th St./P.O. Box 34325/Omaha, NE 68134
402-393-7442

48-
9/10/85

March 22, 1985

Dr. George Shibley
U.S.D.A.-A.P.H.I.S.
Federal Building
Hyattsville, MD 20782

Dear Dr. Shibley, CA

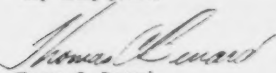
I am enclosing with this letter the general protocol which I follow during the testing of our Pseudorabies Vaccine, Product Code No. 1891.20. I am also enclosing the letter from the state veterinarian in Michigan granting us permission to conduct the tests within his state. Additionally, copies of experimental tables are also submitted.

Previous submissions along with this information should adequately cover the requirements in 9CFR 103.3 (a), (b), (c), (d), (e), and (f). We will notify you prior to any actual shipment of product to , and agree to provide the information if requested as provided for in 9CFR 103.3 (g).

I would also like to request permission to have K.V.S.L. send to adequate challenge material to conduct the tests.

Thank you for your assistance in conducting the test, if you feel a need for additional information, please contact me.

Very truly yours,


Thomas C. Bevard
Director of Regulatory Affairs
Quality Assurance

TCB/tl

Enc.

TechAmerica Group, Inc.

Biologics Corporation
Division of TechAmerica Group, Inc., Animal Health Division
2720 N. 84th St./P.O. Box 34325/Omaha, NE 68134
(402) 393-7440

February 6, 1986

Dr. George Shibley
U.S.D.A. - A.P.H.I.S.
Veterinary Services
Federal Building
Hyattsville, MD 20782

Walter Macke

Dear Dr. Shibley:

As we discussed on the telephone yesterday, we contacted the state veterinarians whose states had been involved in the experimental evaluation of our Pseudorabies Vaccine, Product Code 1891.RC. The contact was by telephone, with follow-up letters (copies attached) to all but Dr. Macke in Minnesota. Dr. Macke is not the state veterinarian, but is responsible for Pseudorabies programs in Minnesota. He stated there was no need to send a letter to his office.

Everyone was comfortable with the organization of the experimental evaluations in their respective states. Nearly all stated that the determining factor was compliance with U.S.D.A. regulations.

Please let me know if you need any further information on this matter.

Sincerely,

Thomas C. Bevard

Thomas C. Bevard
Director of Regulatory Affairs/Quality Control

TCB/lb

TechAmerica Group, Inc.

Biologica Corporation
 (Division of TechAmerica Group, Inc., Animal Health Division)
 2720 N. 84th St./P.O. Box 34325/Omaha, NE 68134
 (402) 395-7441

February 5, 1986

Dr. Charles H. Cole
 State Veterinarian
 Animal Industry Division
 P.O. Box 30017
 Lansing, MI 48909

Dear Dr. Cole:

Approximately one year ago, you granted us permission to conduct testing of a new Pseudorabies vaccine in your state (see enclosed letter).

I would like to inform you that the initial phase of the testing is complete and an U.S.D.A. license (enclosed) for the product has been granted.

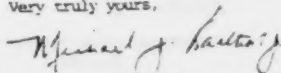
This product, announced at the PRV meeting in Peoria, Illinois in January, has been referred to as the Thymidine Kinase (TK-) negative virus. The virus has been constructed so that a specific gene (TK) necessary for virulence has been permanently deleted. The product, thus, has a margin of safety many orders of magnitude beyond any current products with the protection benefits of a modified-live.

Due to the unique technology used in the development of this virus strain, the U.S.D.A. has given our product an R. O. (Recombinant Organism) designation. While the organism does not contain any recombinant or foreign DNA, the procedures for the development were such that it is classified under this broad category.

As I mentioned above, the first phase of the development is complete and we are now into our second phase. The second phase will conclude with the licensure of a product and associated diagnostics which will allow us to differentiate vaccinates from non-vaccinates. Dr. Thacker will also assist us in the development of this product.

I am enclosing some published papers on our vaccine. If you have additional questions, please feel free to call. Again, thank you for your cooperation.

Very truly yours,



Michael J. Bartkoski, Jr., Ph.D.
 Vice President of Operations

MTB/kjt

Enclosures: Letter of 2/01/86
 U.S.D.A. license
 PRV published papers

TechAmerica Group, Inc.

Biologics Corporation
(Division of TechAmerica Group, Inc., Animal Health Division)
2720 N. 84th St./P.O. Box 34325/Omaha, NE 68134
(402) 393-7446

February 4, 1986

Dr. Paul B. Doby
Division of Meat, Poultry and
Livestock Inspection
State Fairgrounds
Springfield, IL 62706

Dear Dr. Doby:

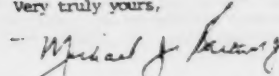
It was a pleasure speaking with you this morning concerning our newly licensed Pseudorabies vaccine. I am enclosing per your request a copy of our release describing the product.

Also enclosed is a copy of the license from U.S.D.A. As mentioned, the product has been given a R. C. designation for a recombinant organism. This is due to the technology involved in generating the deletion in the thymidine kinase (TK) gene.

I am also enclosing some papers describing testing done in the organism.

If I can be of further assistance, please do not hesitate to contact me.

Very truly yours,



Michael J. Bartkoski, Jr., Ph.D.
Vice President of Operations

MJB/kjt

Enclosures: press release
license
articles

T. chAmerica Group, Inc.

Biologics Corporation
(Division of TechAmerica Group, Inc., Animal Health Division)
2720 N. 84th St./P.O. Box 34325/Omaha, NE 68134
(402) 393-7440

February 4, 1986

Dr. Mike Copp
700 N. High School Road
Indianapolis, IN 46224

Dear Dr. Copp:

Biologics Corporation has recently been granted a license for a new Pseudorabies vaccine. The vaccine was announced by the U.S.D.A. at the recent PRV meeting in Peoria, Illinois.

The virus referred to as a Tk- (Thymidine Kinase minus) PRV has had a gene responsible for virulence removed. This has rendered the organism many orders of magnitude safer than any other PRV-MLV on the market.

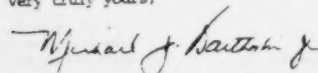
I am enclosing a copy of our U.S.D.A. license. As you can see, the product has been given a R.O. (recombinant organism) designation. Also enclosed is a copy of the patent describing the organism and two published papers.

Due to the technology involved in the deletion of the gene, the organism was classified as a recombinant organism, even though it contains no recombinant or foreign DNA. The U.S.D.A. based this decision on their memorandum 800.68 (enclosed) which requires them (page 3, 4a) to classify any organism as recombinant which utilizes new technology in its development.

We are excited about the licensing of the vaccine as it is the first MLV in which the method of attenuation was specific and deliberate. It signals a new era in the development of MLV vaccines.

If you have additional questions, please feel free to call.

Very truly yours,



Michael J. Bartkoski, Jr., Ph.D.
Vice President of Operations

MJB/kjt

Enclosures: 5

TechnAmerica Group, Inc.

Biogenics Corporation
 Division of TechnAmerica Group, Inc./Animal Health Division
 2720 N. 84th St./P.O. Box 34325/Omaha, NE 68134
 (402) 393-7440

February 4, 1986

Dr. M. H. Lang, Chief
 Division of Animal Husbandry
 Wallace Building
 Des Moines, IA 50319

Dear Dr. Lang:

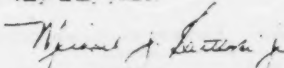
As I mentioned in our phone conversation, we have been granted an U.S.D.A. license for a new *Pseudorabies* vaccine.

The vaccine as was mentioned in the recent PRV meeting in Peoria, Illinois, contains a deletion in a gene-controlling virulence in PRV. This gene, Thymidine Kinase (TK), has thus been rendered non-functional. The resulting organism has been commonly referred to as Tk- minus PRV. Due to the technology involved in removing the Tk gene, the U.S.D.A. has classified the organism as a recombinant organism, even though it contains no recombinant or foreign DNA.

I am enclosing the patent describing the organism, as well as the Veterinary Service Memorandum No. 800.68, which the U.S.D.A. uses to classify the new technology. On page 3, section 4a, the memorandum states "Recombinant derived bacterial or viral seed stocks..." Using the broad interpretation of this regulation, the organism was thus classified.

I hope the enclosed information is sufficient for your files. If any additional questions arise, please feel free to call.

Very truly yours,



Michael J. Bartowski, Jr., Ph.D.
 Vice President of Operations

MLB/kjt

Enclosure



United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Federal Bldg
Hyattsville, MD
20782

November 29, 1985

Mr. Thomas G. Bevard
Biologics Corporation
2720 North 84th Street
Omaha, NE 68134

Dear Mr. Bevard:

This is in response to your submission of October 22, 1985, and our telephone conversations concerning your experimental Pseudorabies Vaccine, Modified Live Virus, Code 1891.R0.

We have completed our review of the immunogenicity study and the report has been filed as satisfactory with the following comments. The immunogenicity study indicates acceptable efficacy as demonstrated by the differences in clinical symptoms and deaths in the vaccinated and control pigs. The misapplication of statistical programs to date can lead to erroneous conclusions. No statistical analyses were necessary for the temperature and weight gain data, but the procedures used were inappropriate even if the conclusions are correct.

Please amend your Outline of Production to include a test for thymidine kinase activity. Include a description of the cell line used and the evaluation process. These results may be submitted on amended VS Forms 14-8.

This is your authorization to submit samples from preclicensing Serials 100-1001, 100-1002, and 100-1003 to National Veterinary Services Laboratories for confirmatory testing.

The stability material has been reviewed and filed. These serials should be retested for potency near the assigned expiration date.

Sincerely,

George P. Shibley, Ph.D.
Chief Staff Microbiologist
Veterinary Biologics Staff
Veterinary Services

DEC 4 1985



United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Federal Bldg
Hyattsville, MD
20782

November 29, 1985

Mr. Thomas C. Bevard
Biologics Corporation
2720 North 84th Street
Omaha, NE 68134

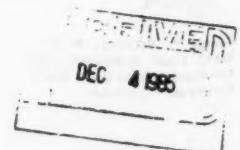
Dear Mr. Bevard:

This is in response to your November 5, 1985, letter requesting additional field trial testing of your experimental Pseudorabies Vaccine, Modified Live Virus, Code 1891.R0. Please note the new product code number for this vaccine.

This is your authorization to conduct these trials in Iowa where written permission has been provided by proper State officials. These trials should be conducted in accordance with the provisions stated in my November 5, 1985, letter.

Sincerely,

George F. Shibley, Ph.D.
Chief Staff Microbiologist
Veterinary Biologics Staff
Veterinary Services



United States
Department of
Agriculture

Animal and
Plant Health
Inspection
Service

Federal Bldg.
Hyattsville, MD
20782

Dr. Bartholomew
Mr. Shadle
Dr. Miller
Dr. Duce
Mr. Shibley

November 4, 1985

Mr. Thomas C. Bevard
Biologics Corporation
2720 North 84th Street
Omaha, NE 68134

Dear Mr. Bevard:

This is in response to your submissions received September 24 and October 17, 1985, with information to support your license application for Pseudorabies Vaccine, Modified Live Virus, Code 1891.80. Please note that the code number of this product has been revised.

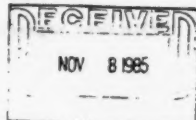
We have completed our review of the Antigen Extinction Report dated August 16, 1985. Due to the wide difference between theoretical and actual use titers, you cannot make any valid conclusions concerning virus titers used in this study.

The report describing the Repeat Antigen Extinction (dated September 20, 1985) was also examined and filed with the following comments. It is again difficult to make assumptions due to the variation between theoretical and actual virus titers used in this study.

The technical problems encountered during the antigen extinction studies should be resolved prior to conducting final efficacy testing.

Sincerely,

G. Shibley
George F. Shibley, Ph.D.
Chief Staff Microbiologist
Veterinary Biologics Staff
Veterinary Services



APPENDIX 10

DEPARTMENT OF Virology and Infectious Diseases
Division of Biochemical Virology • TMS 750-4110



November 12, 1984

Dr. Michael Bartkoski
Biologics Corporation
Div. of TechAmerica Group, Inc.
Animal Health Division
2720 N. 84th Street
Omaha, NE 68134

Dear Mike,

As of this date, I am sending you via Federal Express two bottles (5 ml each) of the tk⁻ deletion mutant [PRV(BUK-dl 3B)] Rab-9-2, made 10/1/83, Lot 2, with a titer of 4.7×10^8 PFU/ml.

Please let me know whether the virus arrives okay.

With best regards,

Sincerely yours,

Saul Kit, Ph. D.
Head, Division of
Biochemical Virology

SK:gb

P.S. Airbill No. 465386261

